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REVIEW ARTICLE

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The key enzyme PYCR1 in proline metabolism: a dual driver of cancer progression and fibrotic remodeling

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ABSTRACT

Pyrroline-5-Carboxylate Reductase 1 (PYCR1), a member of the PYCR family, is a key enzyme in the proline biosynthesis pathway. Notably, PYCR1 was originally identified via genetic disease research, linking its mutations to the occurrence of cutis laxa. PYCR1 contributes to the pathogenesis of malignancies and fibrotic diseases via mechanisms involving metabolic reprogramming, Extracellular Matrix (ECM) remodelling, and redox homeostasis maintenance. PYCR1 upregulation has been reported in multiple malignancies including Hepatocellular Carcinoma (HCC), Lung Cancer (LC), Breast Cancer (BC), Bladder Cancer (BlC), and Gastric Cancer (GC), where it has been shown to promote cancer proliferation, migration, and therapy resistance, correlating significantly with advanced cancer stages and poor prognosis. On the other hand, in fibrotic disorders, PYCR1-mediated proline metabolism has been linked to the progression of pulmonary, myocardial, and cutaneous fibroses. Notably, although PYCR1-targeted small-molecule inhibitors have demonstrated therapeutic potential in preclinical studies, their clinical translation is yet to be validated.

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PYCR1; proline metabolism; fibrosis; cancer; metabolic reprogramming

Introduction

Proline metabolism plays a critical role in cellular pathophysiological functions, especially tumour progression and fibrotic remodelling processes[1–3]. Furthermore, Pyrroline-5-Carboxylate Reductase 1 (PYCR1), a member of the PYCR family, is a key enzyme in the proline synthesis pathway that catalyses the final step of proline generation from glutamate via Pyrroline-5-Carboxylate (P5C) (Figure 1)[4–6]. Owing to its pivotal involvement in multiple diseases, PYCR1 has recently attracted significant attention. For instance, its upregulation has been consistently observed in multiple diseases, including Hepatocellular Carcinoma (HCC), Breast Cancer (BC), Gastric Cancer (GC), Bladder Cancer (BlC), Lung Cancer (LC), and tissue fibrosis[7–13]. Furthermore, PYCR1's mechanisms in promoting cancer and fibrotic disease progression both intracellularly and extracellularly have been documented, highlighting its crucial role involvement in these disorders[14]. As a key enzyme in proline metabolism, PYCR1's functional significance in cancer and fibrotic illnesses offers novel perspectives for understanding the pathological mechanisms underlying these complex conditions. Hitherto, the relationship between PYCR1 and both cancer and fibrotic diseases has not been systematically reviewed. Consequently, besides elucidating PYCR1-driven oncogenesis and fibrogenesis, this comprehensive review could offer innovative therapeutic strategies for related diseases.

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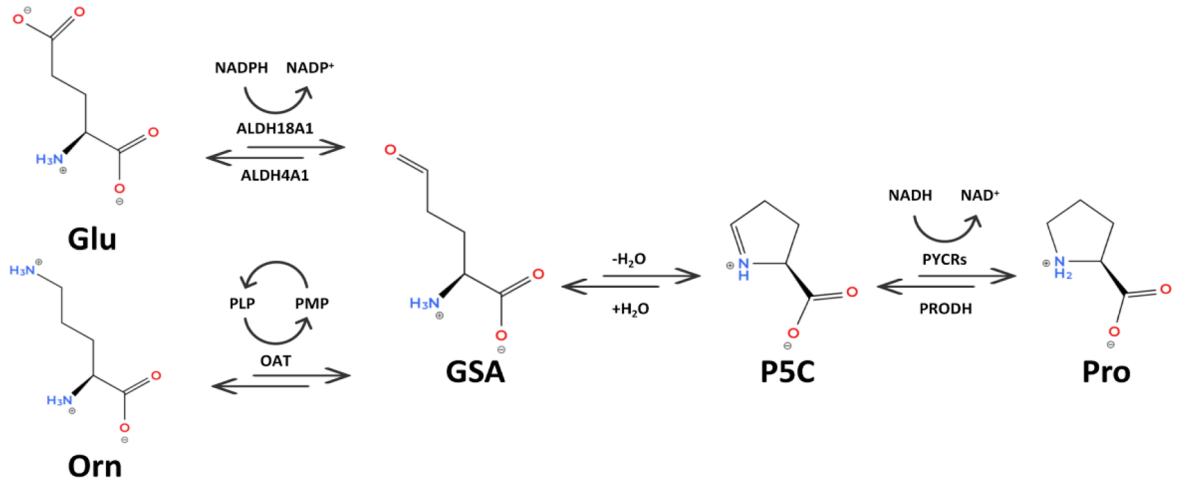

Figure 1. The enzymes and reactions of proline synthesis. Glu: Glutamate; Orn: Ornithine; OAT: Ornithine Aminotransferase; ALDH18A1: Aldehyde Dehydrogenase 18 Family Member A1; ALDH4A1: Aldehyde Dehydrogenase 4 Family Member A1; PLP: pyridoxal-5′-phosphate; PMP: pyridoxamine-5′-phosphate; GSA: Glutamic Acid 5-semialdehyde; P5C: Pyrroline-5-Carboxylate; PYCRs: Pyrroline-5-Carboxylate Reductases; PRODH: Proline Dehydrogenase; Pro: Proline (Adapted from Ref. 15. Figure was created by the authors using MolView software).

Structural features of PYCR1 and other PYCR family members

The three key members of the PYCR family [including PYCR1, PYCR2, and PYCR3 (also designated as PYCRL)] have been critically implicated in proline biosynthesis, cellular redox homeostasis, and metabolic regulation[15]. Whereas PYCR1 and PYCR2 are localised to mitochondria and mainly drive glutamine pathway-mediated proline synthesis, PYCR3/L is cytoplasmically resident and mediates ornithine pathway-dependent proline production[5]. Furthermore, sequence homology analysis revealed that PYCR1 is ~85% similar to PYCR2, with a nearly complete active site conservation. Conversely, PYCR3/L is only 45% homologous to PYCR1/2, with a significant divergence in the active site architecture (Figure 2). Additionally, PYCR1 homologs were identified in yeast, plants, and animals, highlighting its high evolutionary conservation and fundamental role in proline metabolism[16,17].

Genetically, PYCR1 is located on human chromosome 17q25.3 and comprises seven exons. The PYCR1 gene encodes the 319-amino acid mitochondrial matrix enzyme (molecular weight = ~33.4 kDa), which crucially regulates proline synthesis[18,19]. It is also noteworthy that PYCR1 was initially identified via genetic studies on heritable connective tissue disorders, conclusively linking its loss-of-function mutations to Autosomal Recessive Cutis Laxa (ARCL)[20]. Given that proline is the primary substrate for collagen synthesis—a critical dermal elasticity and mechanical resilience determinant, it could impair PYCR1 activity, thus reducing proline availability. This phenomenon, could, in turn, induce defective collagen synthesis and structural destabilisation of the Extracellular Matrix (ECM), manifesting clinically as cutis laxa and premature skin wrinkling[21–23].

Structurally, the PYCR1 protein could assemble into a stable decameric architecture with a "pentamer of dimers" configuration[24], which could be stabilised via the dimerisation interface within its α-helical domain (Figure 3). This unique architecture could further form an annular groove, providing spatially defined binding pockets for cofactors and substrates. Notably, in 2006 Meng et al.[24] reported the first crystal structures of PYCR1, providing initial insights into this protein. However, the low resolution of these structures (3.1 Å) was insufficient to identify the active site. In 2017 Christensen et al.[19] reported high-resolution structures of PYCR1 (1.85 Å) bound to its natural ligands (or analogs) that allowed precise identification of the active site. This protein comprises the N-terminal Rossmann dinucleotide-binding, central dimerisation, and C-terminal α-helical domains[25]. The N-terminal domain has conserved catalytic residues that form the cofactor-binding pocket via alternating β-strands and α-helices. Notably, Asn-56 and Ser-34 form hydrogen bonds with NADH's adenine ring and 2′-phosphate group, respectively, with this region's catalytic core facilitating substrate P5C reduction to proline[19]. On the other hand, the dimerisation domain features an acetylation site (K228) that modulates the PYCR1 oligomerisation status and

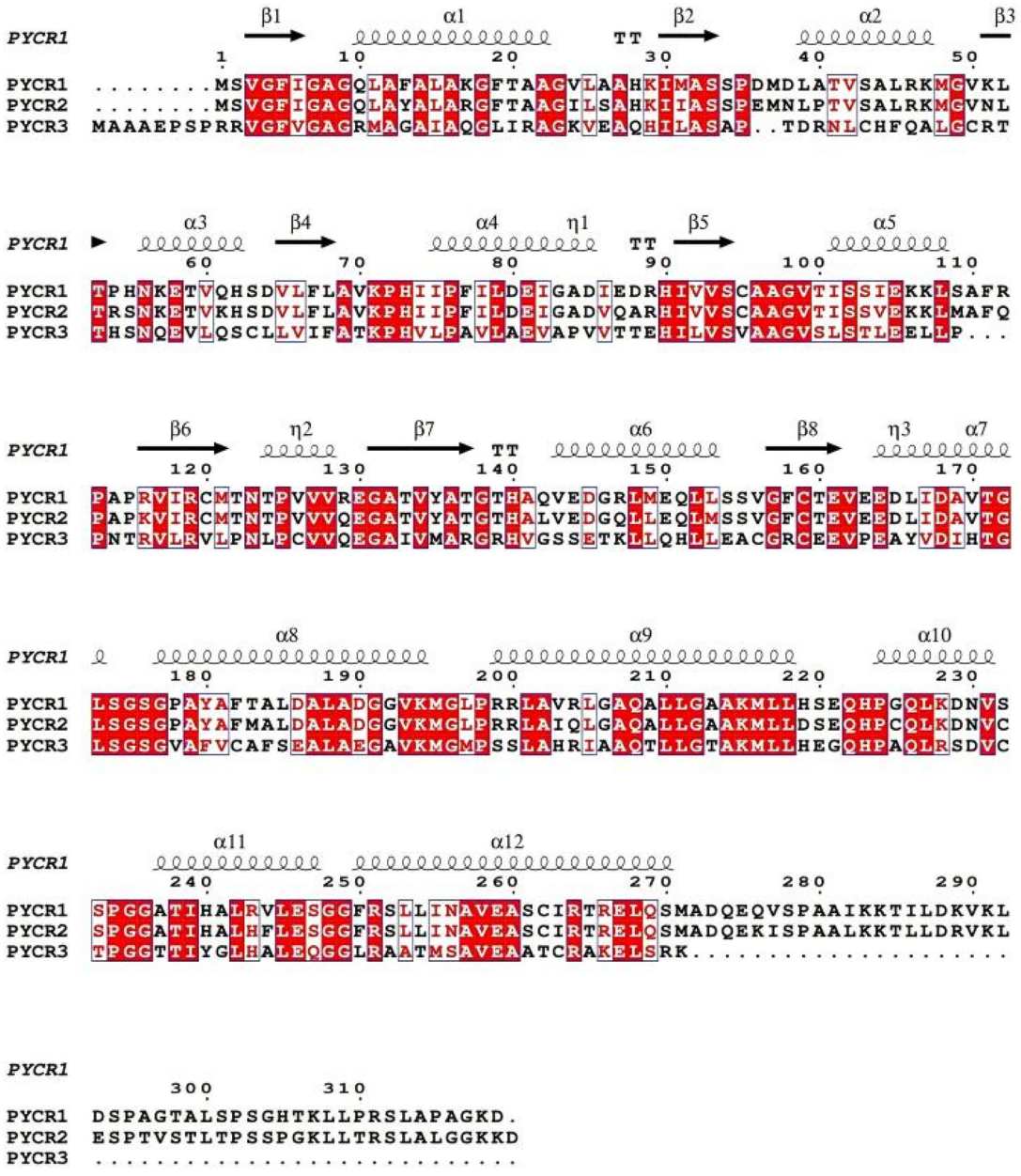

Figure 2. Sequence alignment of PYCR1, PYCR2, and PYCR3 isoforms. Secondary structure elements were obtained from the structure of PYCR1 (PDB ID: 5UAV). Red boxes indicate the conserved sequence (Figure was generated using ESPript 3.0 based on a multiple sequence alignment calculated in DNAMAN).

enzymatic activity. Notably, the hydrogen bonding-mediated Thr-238-(S)-(–)-tetrahydro-2-furoic acid (THFA is a cyclic analogue of proline) amino group interaction could result in catalytic efficiency regulation. Finally, the C-terminal domain regulates oligomerisation via α-helices, thus maintaining the protein conformational stability. Despite the localisation of its active site near the dimer interface, the terminal 19 residues still exhibit a disordered conformation in the crystal structure[15].

PYCR1 and proline metabolism

PYCR1 crucially regulates proline metabolism, which is critical for maintaining intracellular homeostasis and promoting cellular growth and proliferation[26]. Glutamine is the major substrate source for PYCR1-mediated proline metabolism within mitochondria. Following its transportation across the mitochondrial membrane, glutamine is catalysed into glutamate with Glutaminase (GLS). Notably, glutamate

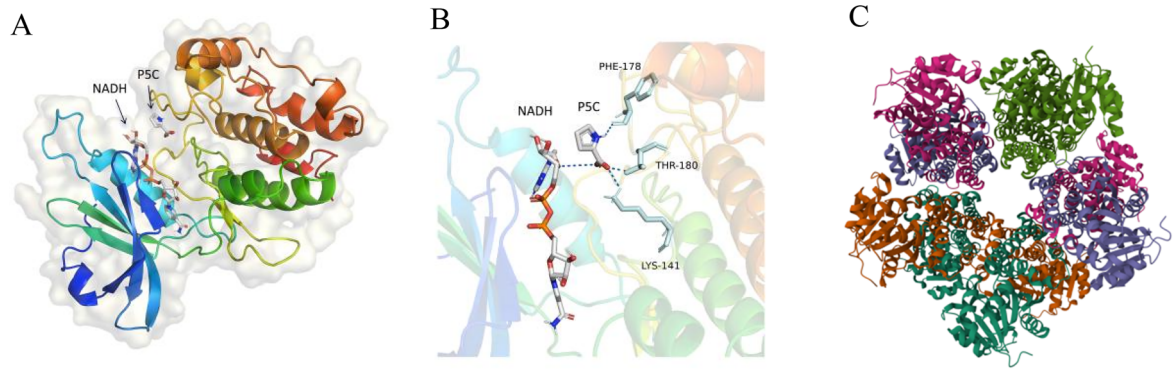

Figure 3. Fold and oligomeric structure of PYCR1. (A) The structure of a PYCR1 protomer showing the protein fold (PDB ID: 5UAV). The polypeptide chain is coloured in a rainbow scheme with dark blue at the N-terminus and orange at the C-terminus. NADH (PubChem CID: 439153) and P5C (PubChem CID: 1196) are shown in colourful sticks. (B) Close-up view of the active site of PYCR1 with NADH and P5C bound. Interactions between P5C, NADH, and and key residues (Phe178, Thr180 and Lys141) are shown as blue dotted lines. Note the active site is fully formed by the presence of the other protomer completing the dimer. (C) Full oligomeric assembly of PYCR1, arranged as a pentamer-of-dimers (decamer). PYCR1: Pyrroline-5-Carboxylate Reductase 1; P5C: Pyrroline-5-Carboxylate (Figure was created by the authors using AutoDock and PyMOL software).

could be either transformed into α-Ketoglutarate (α-KG)—entering the Tricarboxylic Acid (TCA) cycle or undergoing mitochondrial ALDH18A1 (P5CS)-mediated catalysis. This enzymatic reaction consumes ATP and NADPH, thus generating γ-glutamyl-5-semialdehyde (GSA) and $NADP^+$, with the former subsequently undergoing spontaneous cyclisation to form P5C. The final step involves PYCR1 reducing the imine C-atom of P5C utilising NADH as a coenzyme, resulting in proline synthesis[4]. Proline synthesis could also utilise ornithine as a substrate source via its Ornithine Aminotransferase (OAT)-mediated conversion to GSA, thereby entering the proline biosynthetic pathway, which would, in turn, facilitate the cellular conversion of mitochondrial metabolic intermediates into proline, thus meeting endogenous demands (Figure 4A). PYCR1 has high substrate specificity for P5C, ensuring precision in proline biosynthesis via undesired side reaction prevention, thus maintaining an orderly progression of intracellular metabolic processes[16,21]. Proline accounts for ~25% of collagen's amino acid composition. Initially incorporated into collagen peptide chains in its native form, it subsequently undergoes post-translational hydroxylation to form hydroxyproline, and ultimately contributes to the structural stability of the ECM[27,28]. Notably, proline undergoes retrograde catabolism under conditions of proline overload or other initiating factors. During tissue remodelling, injury repair, or fibrotic conditions, hydroxyproline is catalysed by hydroxyproline dehydrogenase (HYPDH) to generate P5C, which subsequently undergoes spontaneous ring-opening to form GSA, ultimately entering the glutamate metabolic pathway[4]. This process is particularly active in metabolically demanding tissues such as the liver and kidneys, establishing a parallel pathway to proline catabolism mediated by proline dehydrogenase (PRODH).

Oncogenically, collagen biosynthesis and post-translational maturation have been established as critical determinants of tumour progression[29,30]. Furthermore, collagen deposition and detached cells contribute to tumour progression by promoting ECM stiffening and proline accumulation and secretion, thereby enhancing neoplastic cell migration[31,32]. Conversely, Matrix Metalloproteinase (MMP)- and collagenase-mediated proteolytic degradation of collagen liberates proline and hydroxyproline, which could be reutilised for *de novo* collagen biosynthesis, thus establishing a feed-forward loop that drives Tumour Microenvironment (TME) remodelling[25,33]. Physiologically, constitutive PYCR1 expression could sustain proline homeostasis, ensuring stable cellular physiological functions via continuous amino acid provision[34]. On the other hand, during embryogenesis or tumorigenesis, proline demand rises significantly in rapidly proliferating cells, necessitating compensatory PYCR1 upregulation to meet biosynthetic requirements[35]. Notably, PYCR1 knockdown may deplete intracellular proline reserves, altering the TME and impairing the neoplastic proliferation capacity. This metabolic perturbation has also been linked with disrupted redox balance and impaired ECM remodelling in malignant cells. Moreover, plant systems have shown the evolutionary conserved dual functionality of proline[36]. Besides its structural role in cell wall

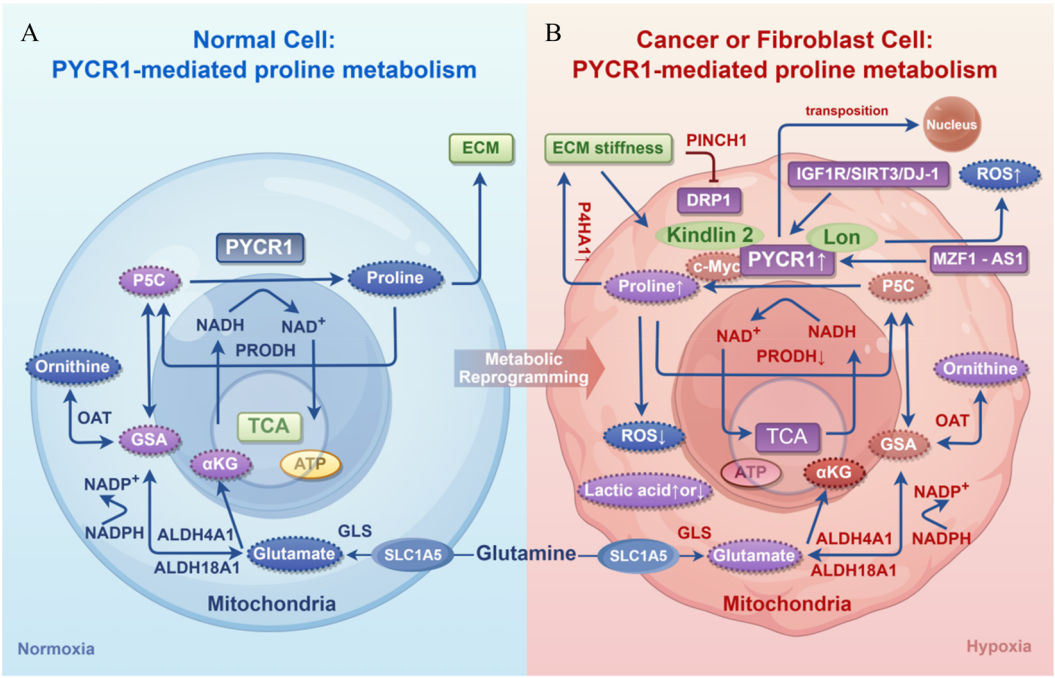

Figure 4. Overview of PYCR1 in Proline Metabolism Under Different Cellular States. (A) PYCR1 is involved in proline metabolism within normal cells. Glutamine is catalysed by GLS to generate glutamate. A portion of glutamate is converted into αKG for entry into the TCA cycle, while another portion is catalysed by ALDH18A1 to produce GSA, a reaction that oxidises NADPH to $NADP^+$. GSA subsequently undergoes spontaneous cyclisation to form P5C. Ultimately, PYCR1 utilises NADH as a coenzyme to reduce the imine C-atom of P5C, thereby generating proline and NAD^+. The synthesised proline can be exported from the cell for ECM synthesis, while NAD^+ may enter the TCA cycle. (B) The impact of PYCR1 upregulation on metabolic reprogramming in disease-relevant cells associated with cancer and fibrotic pathologies. Under pathophysiological conditions, PYCR1 expression is regulated through transcriptional factor interactions, protein complex formations, and microenvironmental stimuli, which collectively contribute to enhanced proline metabolic flux, promoted extracellular matrix synthesis, and augmented cellular oxidative stress tolerance, ultimately supporting adaptive cellular survival mechanisms. PYCR1: Pyrroline-5-Carboxylate Reductase 1; GSA: Glutamic Acid 5-semialdehyde; P5C: Pyrroline-5-Carboxylate; OAT: Ornithine Aminotransferase; αKG: α-Ketoglutarate; TCA: Tricarboxylic Acid Cycle; MZF1-AS1: Myeloid Zinc Finger 1 Antisense RNA 1; ROS: Reactive Oxygen Species; IGF1R: Insulin-like Growth Factor 1 Receptor; DRP1: Dynamin-Related Protein 1; PINCH1: Particularly Interesting New Cysteine-Histidine-Rich Protein 1; PRODH: Proline Dehydrogenase; ALDH18A1: Aldehyde Dehydrogenase 18 Family Member A1; ALDH4A1: Aldehyde Dehydrogenase 4 Family Member A1; P4HA1: Prolyl 4-Hydroxylase Subunit A1; SLC1A5: Solute Carrier Family 1 Member A5; ECM: Extracellular Matrix (Figure was created by the authors using Figdraw software).

proteins, proline also serves as an osmoprotectant and its accumulation has been mechanistically linked to osmotic pressure regulation. Additionally, proline upregulation might mitigate cellular dehydration damage via colligative property modulation.

Proline biosynthesis is a redox-sensitive metabolic pathway in which PYCR1 serves as a key regulator of cellular redox homeostasis[37]. Notably, NADH is often utilised as a coenzyme during the enzymatic reduction of P5C to proline. Furthermore, this reaction might exert dual metabolic functions. Besides regulating proline production, it might also modulate the NADH/NAD^+ ratio, thus maintaining the intracellular redox equilibrium[38]. Moreover, proline synthesis is significantly diminished when PYCR1 activity is pharmacologically inhibited or its expression is genetically downregulated[39]. Consequently, NADH consumption is proportionally reduced, thus dysregulating the NADH/NAD^+ balance. Such redox imbalances could disrupt mitochondrial energetics and impair Reactive Oxygen Species (ROS) detoxification, ultimately compromising cellular metabolic integrity. Furthermore, the NAD^+ generated via PYCR1 catalysis functionally links proline metabolism to the pentose phosphate pathway and *de novo* nucleotide biosynthesis, yielding an integrated metabolic network that synchronises carbon flux distribution and redox cofactor recycling[15]. PYCR1-mediated proline biosynthesis could regulate tumour cell metabolism, promoting NAD^+ regeneration for TCA cycle flux, which, in turn, reduces lactate accumulation, thus sustaining the oncogenic proliferation capacity[40]. Under oxidative stress or hypermetabolic activity conditions,

excessive ROS including superoxide anions and hydrogen peroxide are generated, inducing macromolecular damage and disrupting redox homeostasis, ultimately impairing essential cellular functions[41–43]. This metabolic adaptation could mitigate the potential redox overload resulting from accelerated carbon oxidation in the TCA cycle via PYCR1-driven proline synthesis. Moreover, PYCR1's enzymatic activity has been mechanistically linked to ROS scavenging systems, maintaining redox signalling fidelity and promoting both cancer cell survival and proliferation under metabolic stress[13].

PYCR1's regulatory mechanisms

Multiple mechanisms regulate PYCR1 transcription, translation, and protein activity, thus maintaining normal metabolic functions under physiological conditions. However, these regulatory mechanisms could also exacerbate disease progression under pathological conditions of tumours and fibrotic illnesses, adversely affecting patient prognosis (Figure 4B).

Transcriptional regulation

Various Transcription Factor (TF)-mediated direct and indirect mechanisms have been implicated in PYCR1 transcription. These TFs include those that bind to promoter regions or modulate expression via signal transduction cascades. Specifically, Myeloid Zinc Finger 1 (MZF1) and its antisense RNA1 (MZF1-AS1) have been identified as the transcriptional regulators of PYCR1[44]. Mechanistically, MZF1-AS1 forms RNA-protein complexes, enhancing the interaction between Poly(ADP-Ribose) Polymerase 1 (PARP1) and TF E2F1, thus facilitating E2F1 transactivation—an epigenetic reprogramming cascade that coordinately upregulates both MZF1 and PYCR1. Under stress conditions, MZF1 stimulates the transcriptional activation of pro-inflammatory genes, promoting PYCR1 transcription[45]. Furthermore, the TF c-MYC could directly bind to the promoter region of the PYCR1 gene, thus activating its transcription. Notably, c-MYC knockdown reduced PYCR1 expression in c-MYC-induced Burkitt lymphoma models (P493 cells)[46]. Furthermore, following its activation through the Insulin-Like Growth Factor 1 Receptor (IGF1R)/PI3K/AKT signalling pathway, c-MYC directly upregulated both PYCR1 mRNA and protein expression in Oral Squamous Cell Carcinoma (OSCC)[47]. Additionally, PI3K/AKT signalling pathway activation contributed to 4E-BP1 inactivation, inducing the release of the TF eIF4E, ultimately triggering c-MYC transcription[48]. In GC, PI3K/AKT/c-MYC axis activation has been identified as the primary mechanism underlying PYCR1 upregulation, with PIK3CA mutations and c-MYC amplification linked positively with PYCR1 upregulation[49,50]. Moreover, a positive correlation between c-MYC mRNA expression and PYCR1 protein levels was reported in Luminal B BC[51]. It is also noteworthy that -MYC-driven tumours exhibited enhanced PYCR1's catalytic activity in reducing glutamine-derived P5C to proline, critically supporting cellular redox homeostasis and mitochondrial function.

Epigenetic regulation

PYCR1 expression is regulated at multiple epigenetic levels, including RNA methylation, histone modification, acetylation/deacetylation, ubiquitination, phosphorylation, and non-coding RNAs (ncRNAs)[52]. In normal cells, the CpG islands within the PYCR1 promoter region are often hypermethylated, resulting in PYCR1 transcription suppression[53]. However, intestinal-type GC tissues exhibited significantly lower methylation levels of the PYCR1 promoter at two specific CpG sites (cg20025963 and cg19202384) compared to normal tissues, upregulating gene expression[11]. Meanwhile, the hypomethylation status of the PYCR1 promoter was associated with poorer survival in GC patients, with the methylation levels correlating with tumour Lauren classification and differentiation grade. Additionally, the PYCR1 gene's methylation status was incorporated into the multi-gene prognostic models for Renal Cell Carcinoma (RCC)[54]. The transcriptional coactivators p300 and CREB-Binding Protein (CBP) are structurally and functionally homologous[55]. The p300/CBP complex could induce H3K27 acetylation (H3K27ac) at its promoter region, enhancing PYCR1 transcriptional activity. In GC, p300-mediated H3K27ac modifications synergize with PYCR1 promoter hypomethylation, thus sustaining its overexpression[11]. Furthermore, histone lactylation modifications (e.g. H3K18 lactylation) might influence PYCR1 expression, although the precise regulatory mechanisms require further validation[8]. Additionally, mitochondrial deacetylase SIRT3 could enhance PYCR1 enzymatic activity via K228

deacetylation, promoting BC cell proliferation. Conversely, PYCR1 acetylation could disrupt its decameric structure formation, suppressing both catalytic function and MCF7 cell growth[56]. Cell Cycle Regulatory Interacting Factor 1 (CRIF1) could facilitate PYCR1 deacetylation via SIRT3 activation, restoring tumour cell viability upon CRIF1 knockdown. This molecular cascade highlights CRIF1's mechanism in promoting Non-Small Cell Lung Cancer (NSCLC) progression via SIRT3-mediated PYCR1 deacetylation[57].

In BlC, the E3 ubiquitin ligase BHLHE41 was reported to promote PYCR1 degradation via the ubiquitin-dependent proteasomal pathway[58]. While BHLHE41 overexpression could significantly enhance PYCR1 polyubiquitination, its knockdown might reduce its ubiquitination levels. Consequently, to mediate PYCR1 ubiquitination, BHLHE41 may either directly act as an E3 ligase or recruit E3 ligases. Continuous nuclear localisation of PYCR1 is a prerequisite for its interaction with the TF ELK4. Under hypoxic conditions, IGF1R phosphorylates the tyrosine 135 (Tyr135) residue of PYCR1. The phosphorylated PYCR1 binds directly to ELK4, forming a PYCR1-ELK4 complex, which is then recruited into the promoter regions of ELK4 target genes (KLK10, CNN1), thus driving transcriptional activation[59,60]. Notably, PYCR1 catalyses the oxidation of NADH to NAD^+ within the PYCR1-ELK4 complex, elevating nuclear NAD^+ levels-a cofactor that potentiates the activity of SIRT7[61,62]. SIRT7 activation mediates H3K18 deacetylation, inducing chromatin compaction, which, in turn, suppresses KLK10's pro-apoptotic function. The T238 residue is the other catalytic core of PYCR1[60]. Although the T238 mutant retains an ELK4-binding capacity, its enzymatic inactivation impedes NAD^+ generation—a metabolic disruption phenomenon that might lead to insufficient SIRT7 activation, ultimately promoting tumour cell death.

In HCC, miR-2355-5p binds directly to the 3′-UTR of PYCR1 mRNA, suppressing its translation or promoting degradation, thus negatively regulating PYCR1 expression[63]. The Cancer Genome Atlas (TCGA) data analysis revealed that HCC tissues exhibited significantly lower miR-2355-5p expression levels compared to adjacent non-tumour tissues. Meanwhile, the PYCR1 levels remained elevated, indicating an inverse correlation. In Lung Adenocarcinoma (LUAD), miR-4739 levels correlated negatively with PYCR1 expression and directly targeted PYCR1 through complementary base-pairing[64]. Furthermore, miR-4739 overexpression in HCC827 and A549 cells markedly reduced PYCR1 levels, suppressing tumour cell proliferation and migration. Additionally, the long non-coding RNA LINC00511 could sponge miR-4739, functioning as a competing endogenous RNA (ceRNA). This interaction could alleviate miR-4739-mediated suppression of PYCR1 mRNA, upregulating PYCR1 and ultimately driving tumour growth and metastasis. Additionally, miR-328-3p, miR-4766-5p, and miR-488 were reported to collectively inhibit PYCR1 expression, suppressing lung tumorigenesis[65–67]. It is also noteworthy that miR-150-5p correlated inversely with PYCR1 levels in Nasopharyngeal Carcinoma (NPC) tissues and cells. Moreover, miR-150-5p could suppress PYCR1 expression, thus inhibiting NPC progression[68]. Similarly, miR-1207-5p downregulated PYCR1, suppressing Prostate Cancer (PCa) cell growth while promoting apoptosis[69]. Additionally, miR-621 exerted PYCR1-targeting regulatory effects in Oesophageal Cancer (EC), further expanding PYCR1's miRNA-mediated control network[70].

Protein interaction-mediated regulation

Multiple protein interactions could regulate PYCR1's stability and enzymatic activity. In Colorectal Cancer (CRC) cells (SW480, SW620), PCa cells (DU145), and Oral Cancer (OC) cells (DOK, HSC-3, OECM-1), the mitochondrial chaperone Lon bound directly to PYCR1 and promoted its expression, implying that Lon may stabilise PYCR1 through its chaperone activity[71]. Notably, PYCR1 knockdown almost abolished Lon-mediated upregulation of Epithelial-Mesenchymal Transition (EMT) markers (Snail, Vimentin) in OECM-1 cells. Furthermore, the Lon-PYCR1 complex could induce ROS production via the p38-NF-κB pathway, promoting the release of inflammatory cytokines (TGF-β, IL-6, IL-13, and VEGF-A), thus driving EMT progression, angiogenesis, and M2 macrophage polarisation. This cascade could create an immunosuppressive TME that might promote tumour malignancy.

Kindlin-2, a cell adhesion and signalling protein, could interact with PYCR1 through its F1 and F3 subdomains, facilitating the formation of a stable mitochondrial complex, which might physically shield PYCR1 from proteasomal degradation, thus maintaining its protein stability[13,72]. Furthermore, Kindlin-2 knockdown might significantly reduce PYCR1 protein levels. Kindlin-2-deficient mice exhibited markedly reduced pulmonary tumour burden and mortality rates, as well as reduced collagen

deposition—phenotypic alterations that Kindlin-2 re-expression restored[28,73]. Additionally, TGF-β1 could promote the mitochondrial translocation of Kindlin-2 via Smad signalling pathway activation, a spatial redistribution that might enable Kindlin-2 to complex with mitochondrial PYCR1. The resultant interaction could stabilise PYCR1 protein levels, thus enhancing the proline biosynthesis capacity. Moreover, ECM stiffening might enhance the mitochondrial translocation of Kindlin-2 within the TME, increasing Kindlin-2-PYCR1 complex formation, thus promoting proline biosynthesis and tumour growth[74,75]. The Particularly Interesting New Cysteine-Histidine-Rich Protein 1 (PINCH-1) has been established to control the expression of Dynamin-Related Protein 1 (DRP1), a key regulator of mitochondrial fission. Notably, PINCH-1 deficiency might upregulate DRP1, inducing excessive mitochondrial fragmentation. This pathological fission could disrupt Kindlin-2 mitochondrial trafficking and its interaction with PYCR1, suppressing proline synthesis and tumour cell proliferation. Notably, PYCR1 overexpression might rescue DRP1-mediated mitochondrial morphological abnormalities, highlighting the reversibility of the aforementioned regulatory axis[13,28].

The DJ-1 (encoded by the PARK7 gene) protein, a member of the peptidase C56 protein family, could bind directly to PYCR1 intracellularly, enhancing PYCR1 enzymatic activity, thus augmenting cellular anti-apoptotic capacity and proline biosynthesis[76]. Notably, knocking down DJ-1 or PYCR1 might induce a mitochondrial membrane potential collapse, although through distinct pathways. Whereas DJ-1 maintains mitochondrial integrity through its antioxidant function, PYCR1 might regulate the mitochondrial energy status via proline metabolism, implying that the two proteins could synergistically protect mitochondrial function against oxidative stress-induced damage. Under oxidative stress conditions, DJ-1 and PYCR1 might cooperate to promote cell survival. While knocking down either protein might increase cellular sensitivity to oxidative stress, a dual knockdown does not yield additive effects, indicating their co-localisation within a unified protective pathway. Mechanistically, DJ-1 could stabilise intracellular redox homeostasis via ROS scavenging and potentiation of PYCR1-mediated proline synthesis.

Metabolic stress and microenvironmental regulation

Metabolic stress and other microenvironmental factors could regulate PYCR1 expression and activity via multidimensional mechanisms[77]. Glucose deprivation is a common occurrence in tumour tissues due to their high metabolic rates[78–80]. Under glucose-deprived conditions, GC cells exhibited a concentration- and time-dependent PYCR1 upregulation. This adaptive response could increase intracellular proline levels, enabling cancer cells to counteract nutrient deprivation and sustain proliferation[48]. Notably, PI3K/AKT signalling pathway suppression might significantly reduce both PYCR1 mRNA and protein levels, potentially inducing glucose deprivation intolerance, thus highlighting PI3K/AKT's critical role in mediating PYCR1-dependent metabolic adaptation. Psychological stress-induced adrenaline might activate the β-adrenergic receptor-cAMP-PKA signalling cascade, upregulating MYC and ultimately promoting PYCR1 transcription[46,81]. Subsequently, the PYCR1-synthesised proline activates the cGMP-PKG pathway, enhancing Breast Cancer Stem Cell (BCSC) stemness features[82]. Under hypoxia, IGF1R phosphorylates PYCR1's tyrosine 135 (Tyr135) residue, promoting the formation of the PYCR1-ELK4 complex and thereby supporting proline-dependent cell proliferation[60]. Furthermore, PYCR1 deficiency might disrupt mitochondrial NADH/NAD^+ ratio homeostasis, impairing the TCA cycle function and exacerbating cell death in hypoxic regions[40]. Moreover, proline deprivation triggers uncharged tRNA accumulation, thus activating Gcn2 kinase to phosphorylate eIF2α. This signalling cascade could, in turn, induce ATF4 expression, upregulating PYCR1, ALDH18A1, and the proline transporter SNAT2, ultimately enhancing proline biosynthesis and uptake[83]. On the other hand, restored proline levels could disengage Gcn2-eIF2α signalling, terminating ATF4-mediated PYCR1 activation and creating a negative feedback regulatory loop[33]. Overall, PYCR1 knockdown might reduce intracellular proline levels, while an exogenous supplementation of proline could partially compensate for the functional loss of PYCR1 via alternative pathways[84].

PYCR1 in cancer and fibrotic diseases

Various tumours and fibrotic diseases often feature the upregulation and enhanced enzymatic activity of PYCR1. This protein primarily exerts its pathological effects by maintaining cellular proliferation and

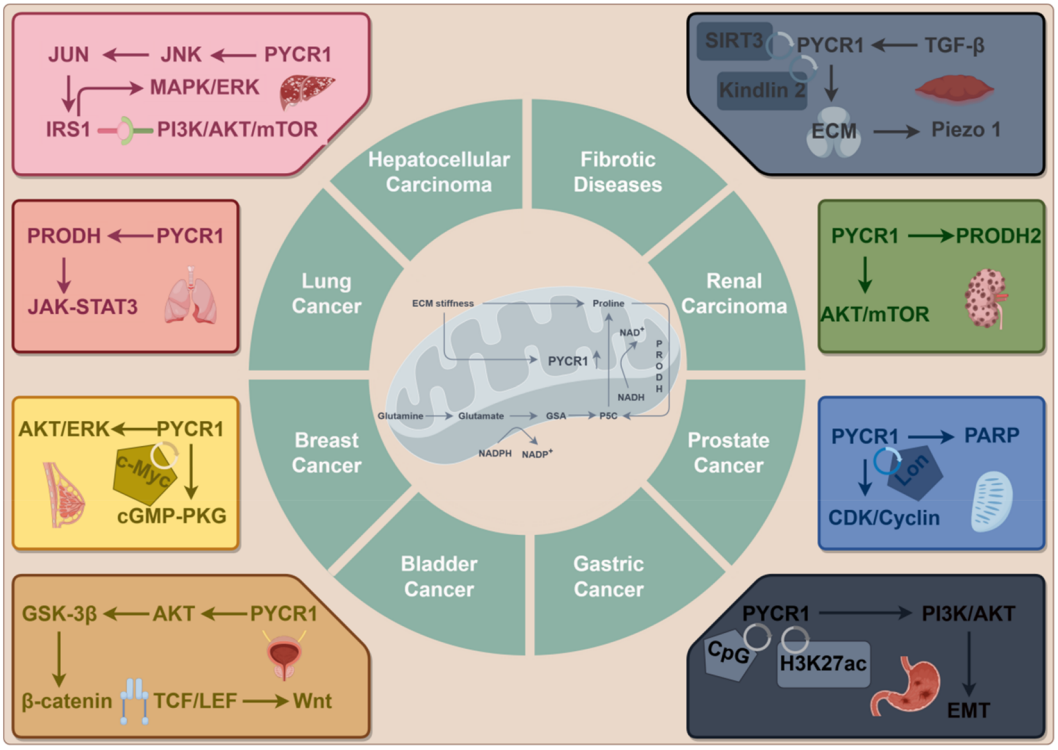

Figure 5. As a key enzyme in proline metabolism, PYCR1 participates in the pathogenesis and progression of various cancers and fibrotic diseases through multiple mechanisms. PYCR1 plays a pivotal role in the pathogenic mechanisms of diverse diseases, thereby underscoring the necessity of targeting PYCR1 as a therapeutic strategy. PYCR1: Pyrroline-5-Carboxylate Reductase 1; GSA: γ-glutamyl-5-semialdehyde; P5C: Pyrroline-5-Carboxylate; PRODH: Proline Dehydrogenase; IRS1: Insulin Receptor Substrate 1; PRODH2: Proline Dehydrogenase 2; ECM: Extracellular Matrix; PARP: Poly(ADP-Ribose) Polymerase; EMT: Epithelial-Mesenchymal Transition (Figure was created by the authors using Figdraw software).

growth, while suppressing apoptosis and autophagy. Furthermore, PYCR1-regulated proline metabolism, collagen synthesis, and redox homeostasis might influence disease progression (Figure 5; Table 1)[15].

PYCR1 and cancer

Compared to adjacent normal tissues, HCC tissues exhibited significantly higher PYCR1 mRNA and protein expression levels, a phenomenon that correlated with younger patients (age <45 years), advanced clinical stages (III/IV), and elevated Alpha-Fetoprotein (AFP) levels[8]. Mechanistically, PYCR1 enriches at the promoter region of the Insulin Receptor Substrate 1 (IRS1) via H3K18 lactylation modification, thereby stimulating IRS1 transcription. The upregulated IRS1 then activates both the PI3K/AKT/mTOR and MAPK/ERK signalling pathways, thus promoting HCC cell proliferation, migration, and metabolic reprogramming[85]. PYCR1 promotes HCC cell migration and invasion via EMT induction, a phenomenon evidenced by E-cadherin/β-catenin downregulation and N-cadherin/Vimentin upregulation[63]. Additionally, PYCR1 was reported to suppress cell apoptosis through dual regulation: upregulating the anti-apoptotic protein Bcl-2 while downregulating pro-apoptotic factors Bax and Caspase3[86]. Mechanistically, PYCR1 knockdown exerts three interconnected effects: (1) Significant reduction in PI3K/AKT/mTOR pathway protein activation; (2) Decreased Bcl-2 expression and intracellular lactate levels; and (3) Impaired glycolysis/proline metabolism. Collectively, these three effects could compromise tumour cell energy production and biosynthetic capacity. Clinically, high PYCR1 expression is an independent prognostic risk factor, specifically associated strongly with shortened Overall Survival (OS) and accelerated disease progression in HCC patients[63,87]. A TCGA cohort analysis revealed that compared to PYCR1-low groups, PYCR1-high patients exhibited significantly worse OS, although the two groups showed no statistical difference in Recurrence-Free Survival (RFS).

Table 1. The pathogenic involvement of PYCR1 in cancer and fibrotic diseases.

Types of diseases	Regulatory mechanism	Signalling pathway	Key alterations	References
Hepatocellular carcinoma	Transcriptional Regulation/ Epigenetic Regulation	PI3K/AKT/mTOR, MAPK/ERK, JNK/IRS1	Cellular proliferation/Migration/ Metabolic reprogramming/ Apoptosis suppression/EMT	8,63,85,86,87
Lung cancer	Epigenetic Regulation/Protein Interaction-Mediated Regulation	JAK-STAT3	Cellular proliferation/Migration	88,89,90,91,92
Breast cancer	Epigenetic Regulation	AKT/ERK, cGMP-PKG	Cellular proliferation/Migration/ECM remodelling/Drug resistance	9,37,51,93,94,95
Bladder cancer	Transcriptional Regulation	AKT/Wnt/β-catenin	Cellular proliferation/Migration/ Apoptosis suppression	11,97,98
Gastric cancer	Transcriptional Regulation/ Epigenetic Regulation	PI3K/AKT	Cellular proliferation/Migration/ Apoptosis suppression	11,48,49,99,100
Prostate cancer	Transcriptional Regulation	CDK/Cyclin, caspase/PARP	Cellular proliferation/Apoptosis suppression	101,102,103
Renal carcinoma	Epigenetic Regulation/Metabolic Stress and Microenvironmental Regulation	AKT/mTOR	Cellular proliferation/Migration/ Drug resistance	107,108,109,110
Pancreatic ductal Adenocarcinoma	Protein Interaction-Mediated Regulation	FTH1/miR-5000-3p	Cellular proliferation/Apoptosis suppression	84,112,115
Colorectal cancer	Protein Interaction-Mediated Regulation	STAT3/p38 MAPK/ NF-κB	Cellular proliferation/Drug resistance /EMT	116
Nasopharyngeal carcinoma	Epigenetic Regulation	miR-150-5p	Cellular proliferation/Metabolic reprogramming	68
Oesophageal carcinoma	Transcriptional Regulation/ Epigenetic Regulation	IGF1R-c-MYC	Cellular proliferation	47,70
Malignant melanoma	Epigenetic Regulation	AKT	Cellular proliferation/Apoptosis suppression	117
Multiple myeloma	Protein Interaction-Mediated Regulation	PRAS40/mTOR/p70 S6, PI3K/AKT/ mTOR, MAPK	Cellular proliferation/Protein synthesis	118
Glioblastoma	Metabolic Stress and Microenvironmental Regulation	VEGF/EGF	Cellular proliferation/Migration/ECM remodelling	113
Pulmonary fibrosis	Protein Interaction-Mediated Regulation	TGF-β	Metabolic reprogramming/ECM remodelling	13,14,28,72,73 123,124,125
Hypertrophic scar/ Keloid/Dermal fibrosis	Metabolic Stress and Microenvironmental Regulation/ Protein Interaction-Mediated Regulation	Piezo1/PINCH1	Metabolic reprogramming/ECM remodelling/Redox imbalance	14
Myocardial fibrosis	Protein Interaction-Mediated Regulation	ROS-p38MAPK	ECM remodelling/Redox imbalance	127,128
Autosomal recessive cutis laxa	Gene mutation	Caspase-3	Mitochondrial damage/Redox imbalance/Increased apoptosis/ Metabolic reprogramming	20,129,130

PYCR1: Pyrroline-5-Carboxylate Reductase 1; IRS1: Insulin Receptor Substrate 1; EMT: Epithelial-Mesenchymal Transition; ECM: Extracellular Matrix; PARP: Poly(ADP-Ribose) Polymerase; IGF1R: Insulin-Like Growth Factor 1 Receptor; EGF: Epidermal Growth Factor; PINCH-1: Particularly Interesting New Cysteine-Histidine-Rich Protein 1.

Compared to normal lung tissues, lung cancer tissues exhibited significantly higher PYCR1 expression levels, particularly in NSCLC[88–90]. Clinically, PYCR1 upregulation correlates significantly with advanced TNM stages (particularly III/IV) and Lymph Node Metastasis (LNM; N-stage)[91]. Mechanistically, PYCR1 activates the JAK-STAT3 pathway, inducing STAT3 phosphorylation and ultimately driving LC cell proliferation, migration, and invasion[92]. Furthermore, PYCR1 could upregulate Cyclin D1 and CDK family proteins (CDK1, CDK4), thereby accelerating cell cycle progression. Conversely, PYCR1 knockout could induce a G1-phase or G2/M-phase arrest, thereby suppressing proliferation. Additionally, PYCR1 could inhibit autophagy-related cell death via ULK1, Beclin1, and LC3 downregulation, thus promoting tumour growth. Prognostically, PYCR1 upregulation correlated significantly with shortened OS in LC patients, highlighting its potential as an independent adverse prognostic factor. Moreover, PYCR1 overexpression correlates with PD-L1-mediated immune evasion via T-cell infiltration suppression.

In a previous study, Cancer-Associated Fibroblasts (CAFs) and the tumour stroma of BC patients exhibited significant PYCR1 upregulation. By catalysing proline synthesis, PYCR1 promotes COL1A1 synthesis, thereby creating a pro-tumour ECM[9,93]. Furthermore, Pyruvate Dehydrogenase (PDH)-derived acetyl-CoA, which epigenetically activates collagen-related gene transcription by increasing H3K27 acetylation, was reported to regulate PYCR1 expression, with EP300 as a critical enzymatic mediator of the process[94]. Functionally, PYCR1 activates AKT/ERK signalling, thus driving BC cell proliferation and invasion. At the same time, it enhances cancer stem cell properties in Triple-Negative Breast Cancer (TNBC) via

proline-induced cGMP-PKG pathway activation, thus facilitating metastasis and recurrence[82,95]. Genomically, PYCR1 upregulation correlates with PIK3CA mutations and c-MYC amplification, whereas its knockdown downregulates Cyclin D1 and CDK4/6, thereby inducing G0/G1 phase arrest and decreasing S-phase cell populations[37,51]. Clinically, PYCR1 overexpression correlates significantly with larger tumour sizes, higher histological grades, aggressive phenotypes, and shorter RFS. Notably, in HER2+ and TNBC subtypes, PYCR1 expression correlated inversely with patient Survival Rates (SRs)[96].

In BlC tissues, significant PYCR1 overexpression correlated positively with advanced tumour stage and histological grade[11]. Clinical sample analyses revealed that compared to normal tissues, BlC tissues exhibited higher PYCR1 mRNA and protein levels[97]. Mechanistically, PYCR1 interacts directly with the Epidermal Growth Factor Receptor (EGFR), activating the downstream PI3K/AKT signalling pathway, thus promoting aerobic glycolysis (as evidenced by increased glucose uptake, lactate production, and ATP generation) and tumour growth[98]. Furthermore, PYCR1 upregulation enhanced AKT phosphorylation, activating the Wnt/β-catenin signalling axis and ultimately driving tumour cell proliferation and invasion. Conversely, PYCR1 knockdown suppressed AKT activity and blocked cell cycle progression. Activated AKT might further phosphorylate downstream targets including GSK-3β, thus inhibiting the pro-apoptotic protein Bad while upregulating the anti-apoptotic protein Bcl-2, with these effects ultimately collectively suppressing apoptosis. Clinically, in BlC, PYCR1-high patients exhibit significantly lower OS rates than PYCR1-low patients, highlighting PYCR1's potential as a prognostic biomarker for adverse outcomes.

Intestinal-type GC demonstrated molecular subtype-specific associations between PYCR1 expression and genomic instability markers including Microsatellite Instability (MSI) and Chromosomal Instability (CIN). Comparative analyses across TCGA and GEO datasets revealed higher PYCR1 mRNA/protein levels in intestinal-type tumours compared to diffuse or mixed subtypes[11]. Along the "Correa Cascade" progression, PYCR1 expression increases progressively from Non-Atrophic Gastritis (NAG) through Chronic Atrophic Gastritis (CAG), Intestinal Metaplasia (IM), Low-Grade Dysplasia (LGD), to High-Grade Dysplasia (HGD), although some studies reported its reduction to gastritis-equivalent levels at invasive carcinoma stages. Nonetheless, other investigations reported sustained PYCR1 upregulation throughout multistep intestinal-type carcinogenesis, indicating its potential as an early driver of malignant transformation[49]. Mechanistically, PYCR1 activates the PI3K/AKT pathway, thus promoting GC cell proliferation, migration, and anti-apoptotic effects. Meanwhile, PYCR1 knockdown reduces the levels of phosphorylated PI3K and AKT and Snail protein, while upregulating E-cadherin, phenomena that collectively suppress tumour invasion and metastasis[48,99]. Clinically, PYCR1 upregulation correlates with advanced TNM stages, poor differentiation, and an elevated Ki-67 index, implying that it might predict shorter OS and Progression-Free Survival (PFS) in GC patients[100]. Notably, survival disparities are often most pronounced among PYCR1-high patients with MSI subtypes[11].

In previous research involving PCa patients, PYCR1 expression correlated positively with the Gleason score, demonstrating a progressive upregulation with increasing tumour malignancy[101,102]. Although PYCR1 expression correlates with Androgen Receptor (AR) signalling activity, its regulatory mechanisms are independent of direct AR activation. Notably, PYCR1 knockdown significantly suppresses PCa cell proliferation and colony formation by downregulating cell cycle regulators (CDK1, CDK2, and Cyclin B1) while upregulating apoptotic proteins (caspase 3 and PARP), thus inducing G2/M phase arrest and apoptosis. Additionally, PYCR1's metabolic function directly impacts cell survival via its substrate-product balance. While the substrate P5C exhibits pro-apoptotic effects, its metabolic product proline shows anti-apoptotic activity. Clinically, PYCR1 upregulation correlates with a poor prognosis in PCa patients and could be an independent prognostic biomarker[103].

Compared to adjacent normal tissues, renal carcinoma tissues have consistently demonstrated higher PYCR1 mRNA and protein levels. Multivariate Cox analyses in various studies confirmed PYCR1 as an independent prognostic factor, with high-expression cohorts exhibiting significantly reduced median OS than low-expression groups[54,104–106]. Mechanistically, PYCR1 enhances tumour immune evasion via glutamine metabolism potentiation, thus accelerating clear cell Renal Carcinoma (ccRC) progression[107]. Under proline-deficient conditions, RCC cells upregulate PYCR1 while downregulating PRODH2 and ALDH4A1, thus sustaining proline biosynthesis and ultimately promoting tumour proliferation[5,77]. Furthermore, PYCR1 promotes RCC proliferation and survival via AKT/mTOR signalling pathway activation, whereas PYCR1 inhibition reduces the phosphorylated AKT and mTOR levels, thus suppressing tumour

progression[108]. Additionally, PYCR1 upregulation correlated with enhanced resistance to therapeutic agents including cyclophosphamide, IC-87114, and MK-2206 in renal cancer cells[109]. Moreover, PYCR1 was proposed as a potential biomarker, with its overexpression correlating significantly with shortened OS, increased metastasis rates, and a poor clinical prognosis in patients[110].

Additionally, PYCR1 upregulation was reported in other malignancies including Pancreatic Ductal Adenocarcinoma (PDAC), CRC, NPC, Oesophageal Squamous Cell Carcinoma (ESCC), malignant melanoma, multiple myeloma, and Glioblastoma (GBM), with its high expression correlating with disease progression[37,47,60,66,68,84,101,111–118]. The oncogenic effects were primarily mediated via proline biosynthesis catalysis, which disrupted NADH/NAD^+ homeostasis, enhancing tumour cell energy metabolism and OS resistance, thus coordinating proliferative, apoptotic, and ECM synthetic processes. Mechanistically, these biological effects were implicated in critical signalling cascades including the JAK/STAT, MAPK/ERK, and PI3K/AKT/mTOR pathways.

PYCR1 and fibrotic diseases

Comparative analyses revealed significantly higher proline concentrations in fibrotic tissues compared to non-fibrotic counterparts[119–121]. Notably, proline plays critical functional roles in collagen biosynthesis and fibrotic regulation, with precise biosynthetic control governing its metabolic homeostasis. Therefore, pharmacological modulation of proline biosynthesis regulators and related signalling pathways presents potential therapeutic avenues for attenuating fibrogenesis progression[13,28,122].

Lung tissues from pulmonary fibrosis patients have consistently shown elevated PYCR1 mRNA and protein expression, highlighting positive correlations with upregulated fibrosis markers (α-SMA and Col1A1)[28,123,124]. Notably, TGF-β1, a key profibrotic cytokine in pulmonary fibrosis, upregulated Kindlin-2 and promoted its mitochondrial translocation in lung fibroblasts, enhancing physical interactions with PYCR1[13,125]. This molecular interplay directly enhanced PYCR1 enzymatic activity, amplifying proline biosynthesis[72]. Furthermore, genetic ablation or knockdown of Kindlin-2 significantly reversed TGF-β1-induced PYCR1 expression, proline synthesis, and collagen production even under strong fibrotic stimuli[73]. Mechanistically, ECM stiffening inhibited DRP1 via integrin signalling mediators (PINCH-1 and Kindlin-2), coordinately increasing Kindlin-2/PYCR1 binding affinity and proline biogenesis[13]. Furthermore, mechanotransduction through the mechanosensitive ion channel Piezo1 activated arginine-proline metabolic circuits, inducing PYCR1 overexpression and subsequent collagen deposition[14].

Murine skin fibrosis models exhibited significantly higher PYCR1 protein expression than normal skin tissues, with co-localisation patterns detected for α-SMA, thus implying functional involvement in fibroblast activation during fibrogenesis[14]. Fibroblasts could respond to ECM stiffening via mechanotransduction pathways, thus determining cellular differentiation trajectories and transcriptional reprogramming[126]. Pathologically, ECM stiffening in fibrotic tissues activated Piezo1 mechanoreceptors, upregulating critical metabolic enzymes including Arginase 1 (Arg1), OAT, and PYCR1. This coordinated metabolic shift potentiated proline biosynthesis, exacerbating ECM rigidity via cross-linked collagen deposition, thus creating a pathological positive-feedback loop.

Notably, PYCR1-mediated proline metabolism has been crucially implicated in mitochondrial homeostasis maintenance, with its enzymatic insufficiency exacerbating oxidative stress, thus promoting cardiomyocyte injury and myocardial fibrosis[127]. In Isoproterenol (ISO)-induced cardiac hypertrophy models, the small-molecule activator 2-APQC upregulated PYCR1 expression via SIRT3 activation, thus potentiating mitochondrial proline metabolism. This metabolic reprogramming promoted ROS scavenging, attenuating ROS-p38MAPK signalling and suppressing fibrotic remodelling[128]. Notably, the cardioprotective effects of 2-APQC were found to be dependent on SIRT3-PYCR1 axis activation, as evidenced by the abolished anti-fibrotic efficacy in SIRT3-knockout models. This protective mechanism contrasts PYCR1's previously reported pro-fibrotic roles, implying tissue-specific regulatory paradigms where microenvironmental indications and signalling networks regulate PYCR1's functional duality.

Mutations in PYCR1 are the primary cause of ARCL, and it is clinically characterised by dermatological laxity, progeroid features (including skin wrinkling and joint hypermobility), and developmental delay[20]. Patients with ARCL present with altered mitochondrial membrane potential and increased apoptosis of dermal fibroblasts under oxidative stress, accompanied by significantly reduced protein expression of

PYCR1 and P5CS[129]. These pathological phenotypes were recapitulated in zebrafish and Xenopus knockdown models, where suppression of PYCR1 orthologs induced significant epidermal developmental defects and elevated apoptosis, with phenotypic analyses demonstrating its essential role in the maintenance of mitochondrial function and cellular survival. Mechanistically, PYCR1 deficiency dysregulates proline metabolism, thereby disrupting mitochondrial redox homeostasis by altering NADH/NAD^+ ratios, leading to membrane depolarisation and ROS accumulation[23]. This metabolic perturbation then drives fibroblast dysfunction and connective tissue maldevelopment[130,131]. In addition, PYCR1 might regulate the phosphorylation activity of various signalling pathways, including PI3K/AKT, mTORC1, and IL-6-JAK-STAT3 cascades[48,107].

PYCR1 inhibitors

Emerging evidence indicates that therapeutic inhibition of PYCR1 may be an attractive strategy for treating cancer and fibrotic diseases (Figure 6; Table 2). In HCC, molecular inhibitors (e.g. shikonin) and gene silencing approaches (siRNA/shRNA) targeting PYCR1 have shown good potential to inhibit tumour growth *in vitro* and *in vivo*[132]. In BC, suppression of the PYCR1 activity by N-Formyl-L-Proline (NFLP) was found to significantly reduce intracellular proline levels, accompanied by increased mitochondrial membrane depolarisation and elevated apoptosis rates[133,134]. Although small-molecule inhibitors, including pargyline and pargyline derivatives, have demonstrated efficacy in inhibiting BC and multiple myeloma in various experimental models, these agents have not been tested clinically[118,135]. It is critical to note that the direct binding of pargyline and its derivatives to PYCR1 has not been structurally characterised through X-ray crystallography. Therefore, their precise inhibitory mechanisms remain unclear. In striking contrast, NFLP has been structurally validated by X-ray crystallography (≤2.0 Å)[134]. NFLP forms a specific hydrogen-bond network with dimer interface residues D229, H223, and V231 of PYCR1 through its formyl and carboxylate groups, inducing a local helical displacement (1 Å) and long-range decamer remodelling, thereby enabling competitive occupancy of the P5C pocket.

Preclinical evidence has suggested that PYCR1 inhibition may enhance chemotherapeutic efficacy in TNBC patients[82]. In a previous study, it was found that 3,5-dichlorophenyl- and 3,5-dibromophenyl derivatives almost completely inhibited the activity of BC PYCR1 at concentrations as low as 1 µmol/L[136]. However, direct experimental evidence demonstrating their direct binding to PYCR1 is lacking; consequently, their inhibitory mechanism remains to be further elucidated. In BlC, exosomes derived from Bone Marrow-derived mesenchymal Stem Cells (BMSCs) loaded with si-PYCR1 (Exo-si-PYCR1) were found to specifically suppress PYCR1 expression in malignant cells, resulting in PI3K/AKT pathway inhibition, significant tumour volume reduction, and enhancing their biocompatibility[98]. In GC, PI3K/AKT inhibitors (e.g. LY294002) or c-MYC-targeted therapies can indirectly regulate the PYCR1 activity, but the specific inhibitors have not been developed[11,46,49]. Using PCa models, it has been demonstrated that PYCR1 suppression via NFLP or genetic silencing approaches significantly attenuates tumour progression[137]. Notably,

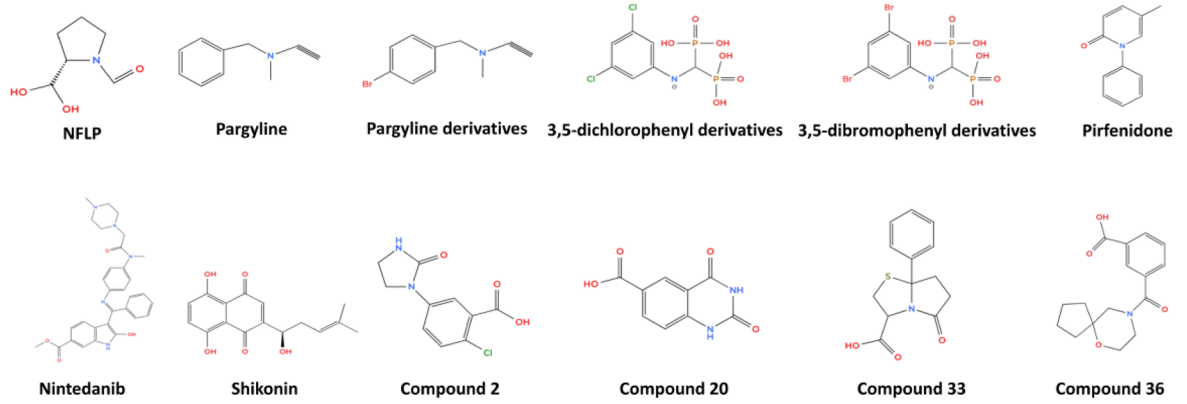

Figure 6. The chemical structure of the main inhibitors of PYCR1. NFLP: N-Formyl-L-Proline (Figure was created by the authors using MolView software).

Table 2. Inhibitors of PYCR1.

Reagent	Inhibitory mechanism	Cells	Inhibitory effect	References
NFLP	Alter the conformation of PYCR1 (validated by X-ray crystallography)	Breast cancer cells	Proline metabolic reprogramming/ Apoptosis/Chemosensitive/Inhibit cell proliferation	133,134
Pargyline and its derivatives	Alter the conformation of PYCR1, Inhibition of PI3K/AKT/mTOR and JNK (unvalidated by X-ray crystallography)	Breast cancer cells/ Multiple myeloma cells/ CAFs	Proline metabolic reprogramming/ Maladaptation to the microenvironment/Inhibit cell proliferation	118,135,139,140,141
3,5-dichlorophenyl-/3,5-dibromophenyl derivatives	Competitively binds to P5C (unvalidated by X-ray crystallography)	Breast cancer cells	Proline metabolic reprogramming/ Cell death	136
Pirfenidone/Nintedanib	Inhibit TGF-β1/Kindlin-2 translocation	Pulmonary fibrotic cells/CAFs	Proline metabolic reprogramming/ ECM remodelling/Maladaptation to the microenvironment	73,139,140,141
Exo-si-PYCR1	Suppress PYCR1 expression	Bladder cancer cells	Metabolic reprogramming/Inhibit cell proliferation	98
Shikonin	Inhibition of PI3K/AKT/mTOR	Liver cancer cells	Inhibit cell proliferation/Apoptosis	132
Compounds 2, 20, 33, and 36	Alter the conformation of PYCR1 (validated by docking-guided X-ray crystallography)	/	/	138

PYCR1: Pyrroline-5-Carboxylate Reductase 1; NFLP: N-Formyl-L-Proline; CAFs: Cancer-Associated Fibroblasts; P5C: Pyrroline-5-Carboxylate; ECM: Extracellular Matrix.

recent studies employing docking-guided X-ray crystallography have discovered a novel class of fragment-based inhibitors[138]. These fragments exhibit structural novelty compared to NFLP, as they demonstrate the ability to block both the P5C substrate pocket and the NADH binding site. Among the four hits (compounds 2, 20, 33, and 36) that showed inhibitory effects on PYCR1 in kinetic assays, compound 33 displayed a lower apparent IC50 value (29 μM) than the current optimal inhibitor NFLP (490 μM). These findings validate the effectiveness of this inhibitor discovery approach and provide a foundation for the development of PYCR1-targeted lead compounds through fragment-to-lead optimisation.

Notably, the clinically approved antifibrotic agents pirfenidone and nintedanib have been shown to alleviate pulmonary fibrosis by suppressing the mitochondrial translocation of Kindlin-2 and its subsequent interaction with PYCR1, thereby reducing pathological proline biosynthesis[73,139–141]. Nevertheless, research on PYCR1 inhibitors has predominantly focused on theoretical frameworks and preclinical studies[118,142], necessitating further research to validate their clinical efficacy and safety profiles.

Discussion and conclusion

In neoplastic cells, PYCR1 promotes tumour progression primarily by enhancing cellular proliferation, invasion, and migration, while concurrently reinforcing metabolic adaptability via coordinated regulation of glucose and proline metabolism. Conversely, in cancer-associated fibroblasts and fibrotic pathologies, PYCR1 promotes collagen biosynthesis by inducing the catalytic conversion of glutamine to proline, thereby providing essential substrates for ECM deposition[9]. This metabolic reprogramming plays a crucial role in the maintenance of mitochondrial redox homeostasis, creating a pro-survival microenvironment that supports cellular viability and tissue adaptation. Moreover, a feedforward loop is established through PYCR1-mediated collagen/ECM production, which reciprocally activates fibroblast-tumour cell crosstalk to amplify protumorigenic signalling and metabolic symbiosis[113]. Therapeutically, PYCR1 inhibition can achieve dual antitumor effects by simultaneously suppressing malignant cell proliferation and stromal collagen generation. Of note, the metabolic effects of PYCR1 vary significantly across cell types and are influenced by the microenvironmental context and need to be further investigated.

Research has demonstrated that PYCR1 promotes NAD^+ generation, which through enzymatic activation of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and Lactate Dehydrogenase (LDH), consequently elevates glycolytic flux and lactate biosynthesis[143,144]. This metabolic shift may activate the Monocarboxylate Transporter 1 (MCT1)-mediated lactate efflux, causing extracellular microenvironment acidification, which triggers the release and activation of MMP and tumour-associated cathepsin B, eventually inducing ECM degradation and metastatic dissemination.[145] In contrast, an alternative regulatory paradigm has been proposed in which PYCR1-mediated modulation of TCA cycle activity can suppress

the net lactate generation[40]. Notably, studies have uncovered that PYCR1 has a dual regulatory role in ROS generation. Administration of 2-APQC was found to upregulate PYCR1 expression via activating SIRT3, resulting in significant ROS reduction[126]. Conversely, genetic ablation of Kindlin-2 decreased PYCR1 levels and elevated ROS production, whereas PYCR1 reconstitution normalises the redox status[72]. PYCR1-mediated proline biosynthesis can mitigate excessive reductive potential accumulation in the TCA cycle, thereby suppressing ROS generation[13]. Paradoxically, the interaction between Lon protease and PYCR1 leads to activation of the p38-NF-κB signalling cascade, subsequently promoting ROS production[68]. This bidirectional regulatory mechanism may be linked to specific disease contexts or microenvironmental states, which calls for further investigations into the context-dependent complexity of PYCR1 functionality and need for further mechanistic investigations.

PYCR1 is commonly upregulated in multiple cancers, and inhibition studies have demonstrated that suppressing PYCR1 significantly inhibits tumour growth. Moreover, high PYCR1 expression is strongly associated with poor prognosis, underscoring its potential as a therapeutic target. However, the development of PYCR1 inhibitors has progressed slowly; currently, only one inhibitor (NFLP) has a validated crystal structure, and no PYCR1-targeted compounds have entered clinical trials. Several major challenges hinder the development of PYCR1 inhibitors: (1) PYCR1 and PYCR2 exhibit high sequence homology, increasing the risk of cross-reactivity and off-target effects. Furthermore, the maintenance mechanism of PYCR1's decameric structure is complex, requiring inhibitors to bind to monomers while excluding interference from multimer conformations, greatly complicating inhibitor design; (2) PYCR1's natural substrate P5C shares structural similarity with intermediates in the proline metabolic network (e.g. ornithine, glutamate). Inhibitors designed based on substrate analogs may interfere with other metabolic enzymes such as OAT and ALDH18A1, potentially causing global disruption of proline anabolism and catabolism. This disruption may induce metabolic toxicity in normal tissues (e.g. liver, kidneys); (3) Currently, there is a lack of real-time monitoring methods for PYCR1 activity, making it difficult to accurately assess inhibitor binding and tissue distribution in animal models. Existing assays rely on intracellular proline levels, which can be influenced by dietary intake and extracellular matrix degradation; (4) Most current studies are based on cell lines and xenograft models, while the pathological role of PYCR1 is closely linked to the tumour microenvironment. Existing models fail to recapitulate key microenvironmental features, leading to discrepancies between preclinical findings and clinical outcomes. Future efforts require breaking through the bottleneck in inhibitor development via structural biology, organoid models, and specific target design.

Collectively, the present results indicate that PYCR1 regulates proline metabolism, collagen formation, cellular proliferation, fibrotic progression, and inhibits oxidative stress. Compared with the normal cells and tissues, its expression is significantly upregulated in cancerous and fibrotic pathological contexts. Notably, PYCR1 modulates the pathogenesis of these diseases via multiple signalling pathways that are mediated via the complex interplay between metabolic reprogramming and epigenetic modifications. Furthermore, the pathogenic mechanisms of PYCR1 and the development of its inhibitors require further investigation. Future research should prioritise targeted strategies that focus on these mechanisms, with a strong emphasis on exploring synergistic combinations of PYCR1-targeted approaches with immunotherapy or chemotherapy to accelerate clinical translation.

Authors contributions

CRediT: **Peng Guo**: Writing – original draft; **Chenchun Wu**: Writing – original draft; **Tong Wang**: Data curation; **Yajuan Song**: Data curation; **Xiaozi Liu**: Writing – review & editing; **Xiang Wang**: Writing – review & editing; **Yuhan Zhu**: Validation, Visualization; **Binyu Song**: Conceptualization, Funding acquisition, Visualization; **Yifu Zhu**: Validation, Visualization; **Juan Zhang**: Validation; **Lei Guo**: Validation; **Rui Tao**: Supervision; **Zhou Yu**: Resources, Software; **Baoqiang Song**: Conceptualization, Funding acquisition, Visualization.

Disclosure statement

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References

1. Bai J, Liu T, Tu B, Yuan M, Shu Z, Fan M, Huo S, Guo Y, Wang L, Wang H, et al. Autophagy loss impedes cancer-associated fibroblast activation via downregulating proline biosynthesis. Autophagy. 2023;19(2):632–643.
2. Wang D, Duan JJ, Guo YF, et al. Targeting the glutamine-arginine-proline metabolism axis in cancer. J Enzyme Inhib Med Chem. 2024;39(1):2367129.
3. Patriarca EJ, Cermola F, D'Aniello C, Fico A, Guardiola O, De Cesare D, Minchiotti G. The multifaceted roles of proline in cell behavior. Front Cell Dev Biol. 2021;9:728576.
4. Vettore LA, Westbrook RL, Tennant DA. Proline metabolism and redox; maintaining a balance in health and disease. Amino Acids. 2021;53(12):1779–1788.
5. De Ingeniis J, Ratnikov B, Richardson AD, Scott DA, Aza-Blanc P, De SK, Kazanov M, Pellecchia M, Ronai Z, Osterman AL, et al. Functional specialization in proline biosynthesis of melanoma. PLoS One. 2012;7(9):e45190.
6. Phang JM, Liu W, Hancock CN, Fischer JW. Proline metabolism and cancer: emerging links to glutamine and collagen. Curr Opin Clin Nutr Metab Care. 2015;18(1):71–77.
7. Li Y, Xu J, Bao P, Wei Z, Pan L, Zhou J, Wang W. Survival and clinicopathological significance of PYCR1 expression in cancer: a meta-analysis. Front Oncol. 2022;12:985613.
8. Wang H, Xu M, Zhang T, et al. PYCR1 promotes liver cancer cell growth and metastasis by regulating IRS1 expression through lactylation modification. Clin Transl Med. 2024;14(10):e70045.
9. Kay EJ, Paterson K, Riera-Domingo C, Sumpton D, Däbritz JHM, Tardito S, Boldrini C, Hernandez-Fernaud JR, Athineos D, Dhayade S, et al. Cancer-associated fibroblasts require proline synthesis by PYCR1 for the deposition of pro-tumorigenic extracellular matrix. Nat Metab. 2022;4(6):693–710.
10. Elia I, Broekaert D, Christen S, Boon R, Radaelli E, Orth MF, Verfaillie C, Grünewald TGP, Fendt S-M. Proline metabolism supports metastasis formation and could be inhibited to selectively target metastasizing cancer cells. Nat Commun. 2017;8(1):15267.
11. Xiao S, Yao X, Ye J, Tian X, Yin Z, Zhou L. Epigenetic modification facilitates proline synthase PYCR1 aberrant expression in gastric cancer. Biochim Biophys Acta Gene Regul Mech. 2022;1865(6):194829.
12. Du S, Sui Y, Ren W, Zhou J, Du C. PYCR1 promotes bladder cancer by affecting the AKT/Wnt/β-catenin signaling. J Bioenerg Biomembr. 2021;53(2):247–258.
13. Chen K, Guo L, Wu C. How signaling pathways link extracellular mechano-environment to proline biosynthesis: a hypothesis: PINCH-1 and kindlin-2 sense mechanical signals from extracellular matrix and link them to proline biosynthesis. Bioessays. 2021;43(9):e2100116.
14. He J, Fang B, Shan S, Li Q. Mechanical stiffness promotes skin fibrosis through Piezo1-mediated arginine and proline metabolism. Cell Death Discov. 2023;9(1):354.
15. Bogner AN, Stiers KM, Tanner JJ. Structure, biochemistry, and gene expression patterns of the proline biosynthetic enzyme pyrroline-5-carboxylate reductase (PYCR), an emerging cancer therapy target. Amino Acids. 2021;53(12):1817–1834.
16. Fichman Y, Gerdes SY, Kovács H, Szabados L, Zilberstein A, Csonka LN. Evolution of proline biosynthesis: enzymology, bioinformatics, genetics, and transcriptional regulation. Biol Rev Camb Philos Soc. 2015;90(4):1065–1099.
17. Stum MG, Tadenev ALD, Seburn KL, Miers KE, Poon PP, McMaster CR, Robinson C, Kane C, Silva KA, Cliften PF, et al. Genetic analysis of Pycr1 and Pycr2 in mice. Genetics. 2021;218(1):iyab048.
18. Hu CA, Bart Williams D, Zhaorigetu S, Khalil S, Wan G, Valle D. Functional genomics and SNP analysis of human genes encoding proline metabolic enzymes. Amino Acids. 2008;35(4):655–664.
19. Christensen EM, Patel SM, Korasick DA, Campbell AC, Krause KL, Becker DF, Tanner JJ. Resolving the cofactor-binding site in the proline biosynthetic enzyme human pyrroline-5-carboxylate reductase 1. J Biol Chem. 2017;292(17):7233–7243.
20. Reversade B, Escande-Beillard N, Dimopoulou A, Fischer B, Chng SC, Li Y, Shboul M, Tham P-Y, Kayserili H, Al-Gazali L, et al. Mutations in PYCR1 cause cutis laxa with progeroid features [published correction appears in Nat Genet. 2022 Feb;54(2):213.]. Nat Genet. 2009;41(9):1016–1021.
21. Gardeitchik T, Mohamed M, Fischer B, Lammens M, Lefeber D, Lace B, Parker M, Kim K-J, Lim BC, Häberle J, et al. Clinical and biochemical features guiding the diagnostics in neurometabolic cutis laxa. Eur J Hum Genet. 2014;22(7):888–895.

22. Dimopoulou A, Fischer B, Gardeitchik T, Schröter P, Kayserili H, Schlack C, Li Y, Brum JM, Barisic I, Castori M, et al. Genotype-phenotype spectrum of PYCR1-related autosomal recessive cutis laxa. Mol Genet Metab. 2013;110(3):352–361.
23. Lin D-S, Yeung C-Y, Liu H-L, Ho C-S, Shu C-H, Chuang C-K, Huang Y-W, Wu T-Y, Huang Z-D, Jian Y-R, et al. A novel mutation in PYCR1 causes an autosomal recessive cutis laxa with premature aging features in a family. Am J Med Genet A. 2011;155A(6):1285–1289.
24. Meng Z, Lou Z, Liu Z, Li M, Zhao X, Bartlam M, Rao Z. Crystal structure of human pyrroline-5-carboxylate reductase. J Mol Biol. 2006;359(5):1364–1377.
25. Phang JM, Liu W, Zabirnyk O. Proline metabolism and microenvironmental stress. Annu Rev Nutr. 2010;30(1): 441–463.
26. Pandhare J, Donald SP, Cooper SK, Phang JM. Regulation and function of proline oxidase under nutrient stress. J Cell Biochem. 2009;107(4):759–768.
27. Phang JM. Proline metabolism in cell regulation and cancer biology: recent advances and hypotheses. Antioxid Redox Signal. 2019;30(4):635–649.
28. Guo L, Cui C, Wang J, Yuan J, Yang Q, Zhang P, Su W, Bao R, Ran J, Wu C, et al. PINCH-1 regulates mitochondrial dynamics to promote proline synthesis and tumor growth. Nat Commun. 2020;11(1):4913.
29. Su H, Karin M. Collagen architecture and signaling orchestrate cancer development. Trends Cancer. 2023;9(9): 764–773.
30. De Martino D, Bravo-Cordero JJ. Collagens in cancer: structural regulators and guardians of cancer progression. Cancer Res. 2023;83(9):1386–1392.
31. Necula L, Matei L, Dragu D, Pitica I, Neagu A, Bleotu C, Diaconu CC, Chivu-Economescu M. Collagen family as promising biomarkers and therapeutic targets in cancer. Int J Mol Sci. 2022;23(20):12415.
32. Pilley SE, Hennequart M, Vandekeere A, Blagih J, Legrave NM, Fendt S-M, Vousden KH, Labuschagne CF. Loss of attachment promotes proline accumulation and excretion in cancer cells. Sci Adv. 2023;9(36):eadh2023.
33. D'Aniello C, Patriarca EJ, Phang JM, Minchiotti G. Proline metabolism in tumor growth and metastatic progression. Front Oncol. 2020;10:776.
34. Geng P, Qin W, Xu G. Proline metabolism in cancer. Amino Acids. 2021;53(12):1769–1777.
35. Li Y, Bie J, Zhao L, Song C, Zhang T, Li M, Yang C, Luo J. SLC25A51 promotes tumor growth through sustaining mitochondria acetylation homeostasis and proline biogenesis. Cell Death Differ. 2023;30(8):1916–1930.
36. Alvarez ME, Savouré A, Szabados L. Proline metabolism as regulatory hub. Trends Plant Sci. 2022;27(1):39–55.
37. Hollinshead KER, Munford H, Eales KL, Bardella C, Li C, Escribano-Gonzalez C, Thakker A, Nonnenmacher Y, Kluckova K, Jeeves M, et al. Oncogenic IDH1 mutations promote enhanced proline synthesis through PYCR1 to support the maintenance of mitochondrial redox homeostasis. Cell Rep. 2018;22(12):3107–3114.
38. Tran DH, Kesavan R, Rion H, Soflaee MH, Solmonson A, Bezwada D, Vu HS, Cai F, Phillips JA, DeBerardinis RJ, et al. Mitochondrial NADP+ is essential for proline biosynthesis during cell growth. Nat Metab. 2021;3(4): 571–585.
39. Zhu J, Schwörer S, Berisa M, Kyung YJ, Ryu KW, Yi J, Jiang X, Cross JR, Thompson CB. Mitochondrial NADP(H) generation is essential for proline biosynthesis. Science. 2021;372(6545):968–972.
40. Westbrook RL, Bridges E, Roberts J, Escribano-Gonzalez C, Eales KL, Vettore LA, Walker PD, Vera-Siguenza E, Rana H, Cuozzo F, et al. Proline synthesis through PYCR1 is required to support cancer cell proliferation and survival in oxygen-limiting conditions. Cell Rep. 2022;38(5):110320.
41. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol. 2004;55(1):373–399.
42. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov. 2013;12(12):931–947.
43. Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch Toxicol. 2023;97(10):2499–2574.
44. Fang E, Wang X, Yang F, Hu A, Wang J, Li D, Song H, Hong M, Guo Y, Liu Y, et al. Therapeutic targeting of MZF1-AS1/PARP1/E2F1 axis inhibits proline synthesis and neuroblastoma progression. Adv Sci. 2019;6(19): 1900581.
45. Powell ND, Sloan EK, Bailey MT, Arevalo JMG, Miller GE, Chen E, Kobor MS, Reader BF, Sheridan JF, Cole SW, et al. Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via β-adrenergic induction of myelopoiesis. Proc Natl Acad Sci U S A. 2013;110(41):16574–16579.
46. Liu W, Le A, Hancock C, Lane AN, Dang CV, Fan TW-M, Phang JM. Reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by oncogenic transcription factor c-MYC. Proc Natl Acad Sci U S A. 2012;109(23):8983–8988.
47. Fang K, Sun M, Leng Z, Chu Y, Zhao Z, Li Z, Zhang Y, Xu A, Zhang Z, Zhang L, et al. Targeting IGF1R signaling enhances the sensitivity of cisplatin by inhibiting proline and arginine metabolism in oesophageal squamous cell carcinoma under hypoxia. J Exp Clin Cancer Res. 2023;42(1):73.
48. Roux PP, Topisirovic I. Signaling pathways involved in the regulation of mRNA translation. Mol Cell Biol. 2018;38(12):e00070-18.

49. Xiao S, Li S, Yuan Z, Zhou L. Pyrroline-5-carboxylate reductase 1 (PYCR1) upregulation contributes to gastric cancer progression and indicates poor survival outcome. Ann Transl Med. 2020;8(15):937–937.
50. Calcagno DQ, Leal MF, Assumpcao PP, Smith MA, Burbano RR. MYC and gastric adenocarcinoma carcinogenesis. World J Gastroenterol. 2008;14(39):5962–5968.
51. Craze ML, Cheung H, Jewa N, Coimbra NDM, Soria D, El-Ansari R, Aleskandarany MA, Wai Cheng K, Diez-Rodriguez M, Nolan CC, et al. MYC regulation of glutamine-proline regulatory axis is key in luminal B breast cancer. Br J Cancer. 2018;118(2):258–265.
52. Comes S, Gagliardi M, Laprano N, Fico A, Cimmino A, Palamidessi A, De Cesare D, De Falco S, Angelini C, Scita G, et al. L-Proline induces a mesenchymal-like invasive program in embryonic stem cells by remodeling H3K9 and H3K36 methylation. Stem Cell Reports. 2013;1(4):307–321.
53. Li H, Ning S, Ghandi M, Kryukov GV, Gopal S, Deik A, Souza A, Pierce K, Keskula P, Hernandez D, et al. The landscape of cancer cell line metabolism. Nat Med. 2019;25(5):850–860.
54. Hu F, Zeng W, Liu X. A gene signature of survival prediction for kidney renal cell carcinoma by multi-omic data analysis. Int J Mol Sci. 2019;20(22):5720.
55. Jin Q, Yu L-R, Wang L, Zhang Z, Kasper LH, Lee J-E, Wang C, Brindle PK, Dent SYR, Ge K, et al. Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation. Embo J. 2011;30(2):249–262.
56. Chen S, Yang X, Yu M, Wang Z, Liu B, Liu M, Liu L, Ren M, Qi H, Zou J, et al. SIRT3 regulates cancer cell proliferation through deacetylation of PYCR1 in proline metabolism. Neoplasia. 2019;21(7):665–675.
57. Wang Q, Xie Z, Li C, Xu C, Ding C, Ju S, Chen J, Cui Z, Chen C, Gu B, et al. CRIF1 promotes the progression of non-small-cell lung cancer by SIRT3- mediated deacetylation of PYCR1. J Mol Histol. 2022;53(4):657–667.
58. Xiao S, Chen J, Wei Y, Song W. BHLHE41 inhibits bladder cancer progression via regulation of PYCR1 stability and thus inactivating PI3K/AKT signaling pathway. Eur J Med Res. 2024;29(1):302.
59. Kaikkonen S, Makkonen H, Rytinki M, Palvimo JJ. SUMOylation can regulate the activity of ETS-like transcription factor 4. Biochim Biophys Acta. 2010;1799(8):555–560.
60. Zheng K, Sha N, Hou G, Leng Z, Zhao Q, Zhang L, He L, Xu M, Jiang Y, Chen T, et al. IGF1R-phosphorylated PYCR1 facilitates ELK4 transcriptional activity and sustains tumor growth under hypoxia. Nat Commun. 2023; 14(1):6117.
61. Singh CK, Chhabra G, Ndiaye MA, Garcia-Peterson LM, Mack NJ, Ahmad N. The role of sirtuins in antioxidant and redox signaling. Antioxid Redox Signal. 2018;28(8):643–661.
62. Fang Q, Liu Q, Song Z, Zhang X, Du Y. A NADH oxidase mimic for catalytic tumor therapy via a deacetylase SIRT7-mediated AKT/GSK3β pathway. Nanoscale. 2024;16(13):6585–6595.
63. Xu Y, Zuo W, Wang X, Zhang Q, Gan X, Tan N, Jia W, Liu J, Li Z, Zhou B, et al. Deciphering the effects of PYCR1 on cell function and its associated mechanism in hepatocellular carcinoma. Int J Biol Sci. 2021;17(9): 2223–2239.
64. Ye L, Wang L, Zeng Y. LINC00511 aggravates the malignancy of lung adenocarcinoma through sponging microRNA miR-4739 to regulate pyrroline-5-carboxylate reductase 1 expression. J Clin Lab Anal. 2022;36(12):e24760.
65. Lu J, Lin J, Zhou Y, Ye K, Fang C. MiR-328-3p inhibits lung adenocarcinoma-genesis by downregulation PYCR1. Biochem Biophys Res Commun. 2021;550:99–106.
66. Wang H, He D. LINC01123 acts as an oncogenic driver in lung adenocarcinoma by regulating the miR-4766-5p/PYCR1 axis. Histol Histopathol. 2023;38(12):1475–1486.
67. Wang D, Wang L, Zhang Y, Yan Z, Liu L, Chen G. PYCR1 promotes the progression of non-small-cell lung cancer under the negative regulation of miR-488. Biomed Pharmacother. 2019;111:588–595.
68. Li Z, Zhou X, Huang J, Xu Z, Xing C, Yang J, Zhou X. miR-150-5p inhibits nasopharyngeal cancer genesis by suppressing PYCR1. Am J Med Sci. 2022. doi:10.1016/j.amjms.2022.06.004.
69. Xu H, He Y, Lin L, Li M, Zhou Z, Yang Y. MiR-1207-5p targets PYCR1 to inhibit the progression of prostate cancer. Biochem Biophys Res Commun. 2021;575:56–64.
70. Qian CJ, Tong YY, Wu LK, Wang YC, Teng XS, Yao J. Circ_0000705 facilitates proline metabolism of esophageal squamous cell carcinoma cells by targeting miR-621/PYCR1 axis. Discov Oncol. 2022;13(1):50.
71. Kuo C-L, Chou H-Y, Chiu Y-C, Cheng AN, Fan C-C, Chang Y-N, Chen C-H, Jiang SS, Chen N-J, Lee AY-L, et al. Mitochondrial oxidative stress by Lon-PYCR1 maintains an immunosuppressive tumor microenvironment that promotes cancer progression and metastasis. Cancer Lett. 2020;474:138–150.
72. Guo L, Cui C, Zhang K, Wang J, Wang Y, Lu Y, Chen K, Yuan J, Xiao G, Tang B, et al. Kindlin-2 links mechano-environment to proline synthesis and tumor growth. Nat Commun. 2019;10(1):845.
73. Zhang P, Wang J, Luo W, Yuan J, Cui C, Guo L, Wu C. Kindlin-2 acts as a key mediator of lung fibroblast activation and pulmonary fibrosis progression. Am J Respir Cell Mol Biol. 2021;65(1):54–69.
74. Guo L, Wu C. Mechano-regulation of proline metabolism and cancer progression by kindlin-2. Mol Cell Oncol. 2019;6(3):1596003.
75. Karna E, Szoka L, Huynh TYL, Palka JA. Proline-dependent regulation of collagen metabolism. Cell Mol Life Sci. 2020;77(10):1911–1918.
76. Yasuda T, Kaji Y, Agatsuma T, Niki T, Arisawa M, Shuto S, Ariga H, Iguchi-Ariga SMM. DJ-1 cooperates with PYCR1 in cell protection against oxidative stress. Biochem Biophys Res Commun. 2013;436(2):289–294.

77. Loayza-Puch F, Rooijers K, Buil LCM, Zijlstra J, Oude Vrielink JF, Lopes R, Ugalde AP, van Breugel P, Hofland I, Wesseling J, et al. Tumour-specific proline vulnerability uncovered by differential ribosome codon reading. Nature. 2016;530(7591):490–494.
78. Kanarek N, Petrova B, Sabatini DM. Dietary modifications for enhanced cancer therapy. Nature. 2020; 579(7800):507–517.
79. Huang Y, Xiong C, Wang C, Deng J, Zuo Z, Wu H, Xiong J, Wu X, Lu H, Hao Q, et al. p53-responsive CMBL reprograms glucose metabolism and suppresses cancer development by destabilizing phosphofructokinase PFKP. Cell Rep. 2023;42(11):113426.
80. Li Z, Sun C, Qin Z. Metabolic reprogramming of cancer-associated fibroblasts and its effect on cancer cell reprogramming. Theranostics. 2021;11(17):8322–8336.
81. Cui B, Luo Y, Tian P, Peng F, Lu J, Yang Y, Su Q, Liu B, Yu J, Luo X, et al. Stress-induced epinephrine enhances lactate dehydrogenase A and promotes breast cancer stem-like cells. J Clin Invest. 2019;129(3):1030–1046.
82. Cui B, He B, Huang Y, Wang C, Luo H, Lu J, Su K, Zhang X, Luo Y, Zhao Z, et al. Pyrroline-5-carboxylate reductase 1 reprograms proline metabolism to drive breast cancer stemness under psychological stress. Cell Death Dis. 2023;14(10):682.
83. D'Aniello C, Fico A, Casalino L, Guardiola O, Di Napoli G, Cermola F, De Cesare D, Tatè R, Cobellis G, Patriarca EJ, et al. A novel autoregulatory loop between the Gcn2-Atf4 pathway and l-Proline metabolism controls stem cell identity. Cell Death Differ. 2015;22(7):1234–1234.
84. Park JM, Su Y-H, Fan C-S, Chen H-H, Qiu Y-K, Chen L-L, Chen H-A, Ramasamy TS, Chang J-S, Huang S-Y, et al. Crosstalk between FTH1 and PYCR1 dysregulates proline metabolism and mediates cell growth in KRAS-mutant pancreatic cancer cells. Exp Mol Med. 2024;56(9):2065–2081.
85. Zhuang J, Song Y, Ye Y, He S, Ma X, Zhang M, Ni J, Wang J, Xia W. PYCR1 interference inhibits cell growth and survival via c-Jun N-terminal kinase/insulin receptor substrate 1 (JNK/IRS1) pathway in hepatocellular cancer. J Transl Med. 2019;17(1):343.
86. Ding Z, Ericksen RE, Escande-Beillard N, Lee QY, Loh A, Denil S, Steckel M, Haegebarth A, Wai Ho TS, Chow P, et al. Metabolic pathway analyses identify proline biosynthesis pathway as a promoter of liver tumorigenesis. J Hepatol. 2020;72(4):725–735.
87. Monge C, Francés R, Marchio A, Pineau P, Desterke C, Mata-Garrido J. Characterization of an activated metabolic transcriptional program in hepatoblastoma tumor cells using scRNA-seq. Int J Mol Sci. 2024;25(23):13044.
88. Cai F, Miao Y, Liu C, Wu T, Shen S, Su X, Shi Y. Pyrroline-5-carboxylate reductase 1 promotes proliferation and inhibits apoptosis in non-small cell lung cancer. Oncol Lett. 2018;15(1):731–740.
89. Yang S, Liu Y, Li M-Y, Ng CSH, Yang S-L, Wang S, Zou C, Dong Y, Du J, Long X, et al. FOXP3 promotes tumor growth and metastasis by activating Wnt/β-catenin signaling pathway and EMT in non-small cell lung cancer. Mol Cancer. 2017;16(1):124.
90. Wang F, Su Q, Li C. Identidication of novel biomarkers in non-small cell lung cancer using machine learning. Sci Rep. 2022;12(1):16693.
91. Zhang L, Zhao X, Wang E, Yang Y, Hu L, Xu H, Zhang B. PYCR1 promotes the malignant progression of lung cancer through the JAK-STAT3 signaling pathway via PRODH-dependent glutamine synthesize. Transl Oncol. 2023;32:101667.
92. Gao Y, Luo L, Xie Y, Zhao Y, Yao J, Liu X. PYCR1 knockdown inhibits the proliferation, migration, and invasion by affecting JAK/STAT signaling pathway in lung adenocarcinoma. Mol Carcinog. 2020;59(5):503–511.
93. Fan G, Yu B, Tang L, Zhu R, Chen J, Zhu Y, Huang H, Zhou L, Liu J, Wang W, et al. TSPAN8+ myofibroblastic cancer-associated fibroblasts promote chemoresistance in patients with breast cancer. Sci Transl Med. 2024;16(741):eadj5705.
94. Ding J, Kuo M-L, Su L, Xue L, Luh F, Zhang H, Wang J, Lin TG, Zhang K, Chu P, et al. Human mitochondrial pyrroline-5-carboxylate reductase 1 promotes invasiveness and impacts survival in breast cancers. Carcinogenesis. 2017;38(5):519–531.
95. Wu SZ, Roden DL, Wang C, et al. Stromal cell diversity associated with immune evasion in human triple-negative breast cancer. Embo J. 2020;39(19):e104063.
96. Saleh SMI, Bertos N, Gruosso T, Gigoux M, Souleimanova M, Zhao H, Omeroglu A, Hallett MT, Park M. Identification of interacting stromal axes in triple-negative breast cancer. Cancer Res. 2017;77(17):4673–4683.
97. Li Z, Liu J, Fu H, Li Y, Liu Q, Song W, Zeng M. SENP3 affects the expression of PYCR1 to promote bladder cancer proliferation and EMT transformation by deSUMOylation of STAT3. Aging. 2022;14(19):8032–8045.
98. Li Z, Jiang Y, Liu J, Fu H, Yang Q, Song W, Li Y. Exosomes from PYCR1 knockdown bone marrow mesenchymal stem inhibits aerobic glycolysis and the growth of bladder cancer cells via regulation of the EGFR/PI3K/AKT pathway. Int J Oncol. 2023;63(1):84.
99. Xiao H, Huang J, Wu H, Li Y, Wang Y. Pro-tumorigenic activity of PYCR1 in gastric cancer through regulating the PI3K/AKT signaling. Heliyon. 2024;10(5):e26883.
100. Harb OA, Elfeky MA, Alabiad MA, Hemeda R, Allam AS, El Hawary AT, Elbaz M, Sharaf AL, Gertallah LM, Abdelaziz AM, et al. PYCR1, BANF1, and STARD8 expression in gastric carcinoma: a clinicopathologic, prognostic, and immunohistochemical study. Appl Immunohistochem Mol Morphol. 2024;32(2):102–110.
101. Zeng T, Zhu L, Liao M, Zhuo W, Yang S, Wu W, Wang D. Knockdown of PYCR1 inhibits cell proliferation and colony formation via cell cycle arrest and apoptosis in prostate cancer. Med Oncol. 2017;34(2):27.

102. Jariwala U, Prescott J, Jia L, Barski A, Pregizer S, Cogan JP, Arasheben A, Tilley WD, Scher HI, Gerald WL, et al. Identification of novel androgen receptor target genes in prostate cancer. Mol Cancer. 2007;6(1):39.
103. Sun L, Tuo Z, Chen X, Wang H, Lyu Z, Li G. Identification of cell differentiation trajectory-related gene signature to reveal the prognostic significance and immune landscape in prostate cancer based on multiomics analysis. Heliyon. 2024;10(6):e27628.
104. Weijin F, Zhibin X, Shengfeng Z, Xiaoli Y, Qijian D, Jiayi L, Qiumei L, Yilong C, Hua M, Deyun L, et al. The clinical significance of PYCR1 expression in renal cell carcinoma. Medicine. 2019;98(28):e16384.
105. Yu Z, Zhan Y, Guo Y, He D. Better prediction of clinical outcome in clear cell renal cell carcinoma based on a 6 metabolism-related gene signature. Sci Rep. 2023;13(1):11490.
106. Wang D, Deng Z, Lu M, Deng K, Li Z, Zhou F. Integrated analysis of the roles of oxidative stress related genes and prognostic value in clear cell renal cell carcinoma. J Cancer Res Clin Oncol. 2023;149(13):11057–11071.
107. Wei X, Zhang X, Wang S, Wang Y, Ji C, Yao L, Song N. PYCR1 regulates glutamine metabolism to construct an immunosuppressive microenvironment for the progression of clear cell renal cell carcinoma. Am J Cancer Res. 2022;12(8):3780–3798.
108. Wang QL, Liu L. PYCR1 is associated with papillary renal cell carcinoma progression. Open Med. 2019;14(1):586–592.
109. Wu Y, Zhang X, Wei X, et al. A mitochondrial dysfunction and oxidative stress pathway-based prognostic signature for clear cell renal cell carcinoma. Oxid Med Cell Longev. 2021;2021:9939331.
110. Xu X, Wang Y, Hu X, Zhu Y, Wang J, Guo J. Effects of PYCR1 on prognosis and immunotherapy plus tyrosine kinase inhibition responsiveness in metastatic renal cell carcinoma patients. Neoplasia. 2023;43:100919.
111. Choi UY, Lee JJ, Park A, Zhu W, Lee H-R, Choi YJ, Yoo J-S, Yu C, Feng P, Gao S-J, et al. Oncogenic human herpesvirus hijacks proline metabolism for tumorigenesis. Proc Natl Acad Sci U S A. 2020;117(14):8083–8093.
112. Wang H, Mao W, Lou W, Jin D, Wu W, Wang D, Kuang T, Rong Y, Xu X, Zhang L, et al. PYCR1: a potential prognostic biomarker in pancreatic ductal adenocarcinoma. J Cancer. 2022;13(5):1501–1511.
113. Zhang M, Bi B, Liu G, Fan X. PYCR1 expresses in cancer-associated fibroblasts and accelerates the progression of C6 glioblastoma. Histol Histopathol. 2025;40(1):89–100.
114. Zhou B, Mai Z, Ye Y, Song Y, Zhang M, Yang X, Xia W, Qiu X. The role of PYCR1 in inhibiting 5-fluorouracil-induced ferroptosis and apoptosis through SLC25A10 in colorectal cancer. Hum Cell. 2022;35(6):1900–1911.
115. Olivares O, Mayers JR, Gouirand V, Torrence ME, Gicquel T, Borge L, Lac S, Roques J, Lavaut M-N, Berthezène P, et al. Collagen-derived proline promotes pancreatic ductal adenocarcinoma cell survival under nutrient limited conditions. Nat Commun. 2017;8(1):16031.
116. Yan K, Xu X, Wu T, Li J, Cao G, Li Y, Ji Z. Knockdown of PYCR1 inhibits proliferation, drug resistance and EMT in colorectal cancer cells by regulating STAT3-Mediated p38 MAPK and NF-κB signalling pathway. Biochem Biophys Res Commun. 2019;520(2):486–491.
117. Ye Y, Wu Y, Wang J. Pyrroline-5-carboxylate reductase 1 promotes cell proliferation via inhibiting apoptosis in human malignant melanoma. Cancer Manag Res. 2018;10:6399–6407.
118. Oudaert I, Satilmis H, Vlummens P, De Brouwer W, Maes A, Hose D, De Bruyne E, Ghesquière B, Vanderkerken K, De Veirman K, et al. Pyrroline-5-carboxylate reductase 1: a novel target for sensitizing multiple myeloma cells to bortezomib by inhibition of PRAS40-mediated protein synthesis. J Exp Clin Cancer Res. 2022;41(1):45.
119. Guzy R, Redente EF. Kindlin for the fire: targeting proline synthesis to extinguish matrix production in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2021;65(1):4–5.
120. Yoon I, Kim S, Cho M, You KA, Son J, Lee C, Suh JH, Bae D-J, Kim JM, Oh S, et al. Control of fibrosis with enhanced safety via asymmetric inhibition of prolyl-tRNA synthetase 1. EMBO Mol Med. 2023;15(7):e16940.
121. Song S, Fu Z, Guan R, Zhao J, Yang P, Li Y, Yin H, Lai Y, Gong G, Zhao S, et al. Intracellular hydroxyproline imprinting following resolution of bleomycin-induced pulmonary fibrosis. Eur Respir J. 2022;59(5):2100864.
122. Guo M, Liu D, Jiang Y, Chen W, Zhao L, Bao D, Li Y, Distler JHW, Zhu H. Serum metabolomic profiling reveals potential biomarkers in systemic sclerosis. Metabolism. 2023;144:155587.
123. Shan B, Guo C, Zhou H, Chen J. Tanshinone IIA alleviates pulmonary fibrosis by modulating glutamine metabolic reprogramming based on [U-13C5]-glutamine metabolic flux analysis. J Adv Res. 2025;70:531–544.
124. Ding X, Liu H, Xu Q, Ji T, Chen R, Liu Z, Dai J. Shared biomarkers and mechanisms in idiopathic pulmonary fibrosis and non-small cell lung cancer. Int Immunopharmacol. 2024;134:112162.
125. Schwörer S, Berisa M, Violante S, Qin W, Zhu J, Hendrickson RC, Cross JR, Thompson CB. Proline biosynthesis is a vent for TGFβ-induced mitochondrial redox stress. Embo J. 2020;39(8):e103334.
126. Wu J, Subbaiah KCV, Xie LH, Jiang F, Khor E-S, Mickelsen D, Myers JR, Tang WHW, Yao P. Glutamyl-prolyl-tRNA synthetase regulates proline-rich pro-fibrotic protein synthesis during cardiac fibrosis. Circ Res. 2020;127(6):827–846.
127. Peng F, Liao M, Jin W, Liu W, Li Z, Fan Z, Zou L, Chen S, Zhu L, Zhao Q, et al. 2-APQC, a small-molecule activator of Sirtuin-3 (SIRT3), alleviates myocardial hypertrophy and fibrosis by regulating mitochondrial homeostasis. Signal Transduct Target Ther. 2024;9(1):133.
128. Romero-Becerra R, Santamans AM, Folgueira C, Sabio G. p38 MAPK pathway in the heart: new insights in health and disease. Int J Mol Sci. 2020;21(19):7412.

129. Kuo M-L, Lee MB-E, Tang M, den Besten W, Hu S, Sweredoski MJ, Hess S, Chou C-M, Changou CA, Su M, et al. PYCR1 and PYCR2 interact and collaborate with RRM2B to protect cells from overt oxidative stress. Sci Rep. 2016;6(1):18846.
130. Scherrer DZ, Baptista MB, Matos AH, Maurer-Morelli CV, Steiner CE. Mutations in PYCR1 gene in three families with autosomal recessive cutis laxa, type 2. Eur J Med Genet. 2013;56(6):336–339.
131. Ezzo M, Hinz B. Novel approaches to target fibroblast mechanotransduction in fibroproliferative diseases. Pharmacol Ther. 2023;250:108528.
132. Zhang J, Shang L, Jiang W, Wu W. Shikonin induces apoptosis and autophagy via downregulation of pyrroline-5-carboxylate reductase1 in hepatocellular carcinoma cells. Bioengineered. 2022;13(3):7904–7918.
133. Milne K, Sun J, Zaal EA, Mowat J, Celie PHN, Fish A, Berkers CR, Forlani G, Loayza-Puch F, Jamieson C, et al. A fragment-like approach to PYCR1 inhibition. Bioorg Med Chem Lett. 2019;29(18):2626–2631.
134. Christensen EM, Bogner AN, Vandekeere A, Tam GS, Patel SM, Becker DF, Fendt S-M, Tanner JJ. In crystallo screening for proline analog inhibitors of the proline cycle enzyme PYCR1. J Biol Chem. 2020;295(52):18316–18327.
135. She Y, Mao A, Li F, Wei X. P5CR1 protein expression and the effect of gene-silencing on lung adenocarcinoma. PeerJ. 2019;7:e6934.
136. Forlani G, Sabbioni G, Ragno D, Petrollino D, Borgatti M. Phenyl-substituted aminomethylene-bisphosphonates inhibit human P5C reductase and show antiproliferative activity against proline-hyperproducing tumour cells. J Enzyme Inhib Med Chem. 2021;36(1):1248–1257.
137. Luo Y, Yu J, Lin Z, Wang X, Zhao J, Liu X, Qin W, Xu G. Metabolic characterization of sphere-derived prostate cancer stem cells reveals aberrant urea cycle in stemness maintenance. Int J Cancer. 2024;155(4):742–755.
138. Meeks KR, Ji J, Protopopov MV, Tarkhanova OO, Moroz YS, Tanner JJ. Novel fragment inhibitors of PYCR1 from docking-guided x-ray crystallography. J Chem Inf Model. 2024;64(5):1704–1718.
139. Ahluwalia N, Shea BS, Tager AM. New therapeutic targets in idiopathic pulmonary fibrosis. Aiming to rein in runaway wound-healing responses. Am J Respir Crit Care Med. 2014;190(8):867–878.
140. Raghu G. Idiopathic pulmonary fibrosis: lessons from clinical trials over the past 25 years. Eur Respir J. 2017;50(4):1701209.
141. Raghu G, Remy-Jardin M, Richeldi L, Thomson CC, Inoue Y, Johkoh T, Kreuter M, Lynch DA, Maher TM, Martinez FJ, et al. Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an official ATS/ERS/JRS/ALAT clinical practice guideline. Am J Respir Crit Care Med. 2022;205(9):e18–e47.
142. Meeks KR, Bogner AN, Tanner JJ. Screening a knowledge-based library of low molecular weight compounds against the proline biosynthetic enzyme 1-pyrroline-5-carboxylate 1 (PYCR1). Protein Sci. 2024;33(7):e5072.
143. Tan B, Young DA, Lu Z-H, Wang T, Meier TI, Shepard RL, Roth K, Zhai Y, Huss K, Kuo M-S, et al. Pharmacological inhibition of nicotinamide phosphoribosyltransferase (NAMPT), an enzyme essential for NAD+ biosynthesis, in human cancer cells: metabolic basis and potential clinical implications. J Biol Chem. 2013;288(5):3500–3511.
144. Yamamoto M, Inohara H, Nakagawa T. Targeting metabolic pathways for head and neck cancers therapeutics. Cancer Metastasis Rev. 2017;36(3):503–514.
145. Dhup S, Dadhich RK, Porporato PE, Sonveaux P. Multiple biological activities of lactic acid in cancer: influences on tumor growth, angiogenesis and metastasis. Curr Pharm Des. 2012;18(10):1319–1330.